From
DEPARTMENT OF MICROBIOLOGY, TUMOR AND CELL BIOLOGY
Karolinska Institutet, Stockholm, Sweden

IMPACT OF INTESTINAL WORMS ON DISTAL IMMUNE RESPONSE AND CONTROL OF CO-INFECTIONS

Xiaogang Feng
冯小刚

Stockholm 2018

Published by Karolinska Institutet.
Printed by E-print AB, 2018

ISBN 978-91-7831-159-0

Impact of intestinal worms on distal immune response and control of co-infections
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Xiaogang Feng
冯小刚

Principal Supervisor:
Associate Professor Susanne Nylén
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Co-supervisor(s):
Professor Mats Wahlgren
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Professor Martin Rottenberg
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Associate Professor Antonio Gigliotti Rothfuchs
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology
Fill in

Opponent:
Senior Lecturer Marika Kullberg
University of York
Department of Biology and Hull York Medical School

Examination Board:
Associate Professor Magnus Åbrink
Swedish University of Agricultural Sciences
Department of Biomedicine &Veterinary public Health

Professor Carmen Fernández
Stockholm University
Department of Molecular Biosciences
The Wenner-Gren Institute

Associate Professor Anna Smed Sörensen
Karolinska Institutet
Department of Medicine, Solna (MedS), K2
Fill in

ABSTRACT

Parasitic worm infections have been suggested to impair control of secondary infections and vaccine efficacy. However, the experimental data regarding the capacity of intestinal nematodes to modulate host immune responses was lacking and the mechanism underlying dampened immune responses, particularly those distal to the gut, incompletely understood.

In this thesis, we investigated the effect of the intestinal nematode *Heligmosomoides polygyrus* on the immune response to BCG infection/immunization. We found that *H. polygyrus* infection impaired $CD4^+$ T cell priming in both spleen and in lymph node distal to the site of the worm infection and reduced the recall immune response, measured as delayed-type hypersensitivity (DTH) to PPD in the skin. Furthermore, products released by the worms such as the excretory-secretory products from *H. polygyrus* (HES), were found to dampen the expansion of mycobacteria-specific $CD4^+$ T cells both *in vitro* and when administered at the site of BCG injection.

We found that dampened immune responses were not primarily due to dissemination of regulatory immune responses induced by the intestinal worm. If molecules released by the worms can disseminate and contribute to immune suppression at distal sites is however not clear.

Importantly, we found that the lymph nodes (LNs) distal to the intestinal worm infection become atrophic and do not reach the same cellularity as worm-free mice upon subsequent BCG infection in the skin. In the smaller LN of worm-infected mice, all lymphocyte populations declined and the composition of lymphocyte subpopulations were found to be altered, seen as a decreased T/B lymphocyte ratio and increased CD4/CD8 T cell ratio. Underlying this phenomenon was the recruitment of lymphocytes to the draining mesenteric LN (mLN). In particular, large numbers of naïve lymphocytes were trapped in the mLN during the chronic infection, which over time resulted in a re-distribution of the lymphocyte pool. De-worming was found to recover the cellularity of distal LN and in turn mend the response to BCG measured in the LN draining the site of injection.

Collectively, our findings show that chronic nematode infection causes a paucity of lymphocytes in peripheral LN, which acts to impair the immune response capacity to the subsequent infection.

LIST OF SCIENTIFIC PAPERS

I. Katja Obieglo*, **Xiaogang Feng***, Vishnu Priya Bollampalli*, Isabel Dellacasa-lindberg, Cajsa Classon, Markus Österblad, Helena Helmby, James P. Hewitson, Rick M. Maizels, Antonio Gigliotti Rothfuchs and Susanne Nylén. Chronic gastrointestinal nematode infection mutes immune responses to mycobacterial infection distal to the gut. J immunol 2016; 196:2262-2271 *Equal contribution

II. **Xiaogang Feng**, Cajsa Classon, Graciela Terán, Yunlong Yang, Lei Li, Sherwin Chan, Ulf Ribacke, Antonio Gigliotti Rothfuchs, Jonathan Coquet and Susanne Nylén. Atrophy of skin-draining lymph nodes predisposes for impaired immune responses to secondary infection in mice with chronic intestinal nematode infection. Plos Pathog 2018; 14(5): e1007008 https://doi.org/10.1371/journal.ppat.1007008

III. Cajsa Classon, **Xiaogang Feng**, Liv Eidsmo, Susanne Nylen. Intestinal nematode infection exacerbates experimental visceral leishmniasis. (Manuscript)

PUBLICATIONS NOT INCLUDED IN THESIS

I. Ting Chen, Christopher A. Tibbitt, **Xiaogang Feng**, Julian M. Stark, Leona Rohrbeck, Lisa Rausch, Saikiran K. Sedimbi, Mikael C. I. Karlsson, Bart N. Lambrecht, Gunilla B. Karlsson Hedestam, Rudi W. Hendriks, Benedict J. Chambers, Susanne Nylén and Jonathan M. Coquet. PPAR-γ promotes type 2 immune responses in allergy and nematode infection. Science Immunology 2017 2(9): eaal5196

II. Vishnu Priya Bollampalli, Lívia Harumi Yamashiro, **Xiaogang Feng**, Damiën Bierschenk, Yu Gao, Hans Blom, Birgitta Henriques-Normark, Susanne Nylén, Antonio Gigliotti Rothfuchs. BCG Skin Infection Triggers IL-1R-MyD88-Dependent Migration of $EpCAM^{low}$ $CD11b^{high}$ Skin Dendritic cells to Draining Lymph Node During CD4+ T-Cell Priming. Plos Pathog 2015; 11(10): e1005206 https://doi.org/10.1371/journal.ppat.1005206

CONTENTS

LIST OF ABBREVIATIONS

AAMs	Alternatively activated macrophage
Ag	Antigen
aLNs	Axilary Lymph nodes
APCs	Antigen presenting cells
BCG	Bacillus Calmette Guérin
BECs	Blood endothelial cells
CAMs	Classical activated macrophages
CCL	Chemokine (C-C motif) ligand
CD	Cluster of differentiation
DCs	Dendritic cells
DTH	Delayed-type hypersensitivity
ELISA	Enzyme-linked immunoSorbent Assay
EVs	Extracellular vesicles
FACS	Flow cytometry
FDCs	Follicular dendritic cells
FEC	Formal-ether concentration
FLOTAC	Floatation based technique
Foxp3	Forkhead box P3
FRCs	Fibroblast reticular cells
GATA3	Gata transcription factor 3
GI	Gastrointestinal
H. polygyrus	*Heligmosomoides polygyrus*
HES	*H. polygyrus* excretory secretory molecules
HEVs	High endothelial vessels
IBD	Inflammatory bowel disease
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ILCs	Innate lymphoid cells
iLNs	Inguinal lymph nodes

iNOS	Inducible nitric oxide synthase
L3	The 3rd stage of larvae
LEC	Lymphatic endothelial cells
LNs	Lymph nodes
MALT	Mucosal-associated lymphoid tissue
mLNs	Mesenteric lymph nodes
N. brasiliensis	*Nippostronglus brasiliensis*
NKTs	Natural killer T cells
NO	Nitric oxide
NTD	Neglected tropical disease
P25-TCR Tg	Mycobacteria Ag85 TCR transgenic
pLNs	Popliteal lymph nodes
PPD	Purified protein derivative of *M. tuberculosis*
qPCR	Real-time polymerase chain reaction
S1PR	Sphingosine-1-phosphate receptor
SALT	Skin-associated lymphoid tissue
SCS	Subscapular sinus
STH	Soil-transmitted helminthasis
T. muris	*Trichuris muris*
TB	Tuberculosis
TCR	T cell receptor
Tg	Transgenic
TGF-β	Transfroming growth factor beta 1
TGM	TGF-β mimic
Th	T helper
TNF	Tumor necrosis factor
Treg	Regulatory T cells
TSLP	Thymic stromal lymphopoietin
WHO	World Health Organization

1 INTRODUCTION

1.1 Human Worm Infections

1.1.1 Human Helminthiasis and Soil-Transmitted Helminths (STH)

Helminthiases are a group of neglected tropical diseases (NTDs) caused by worms, also known as helminths (from the Greek *helmins*). The important impact of helminthiasis on human health was recognized in the "The Wormy World", a publication written by Norman Stoll in 1947 (Stoll, 1947). Worm-infections are common diseases and typical symptoms are diarrhea, abdominal pain, general malaise and weakness (Bellagio, 2017).

Parasitic worms typically cause limited damage to their host. Many worms establish chronic infection and survive for many years in their host. However, helminth infections are rarely life threatening since parasitic worms depend on the host for sustenance and reproduction (Lacey, 1982). Helminths of clinical relevance to humans belongs to two phyla, roundworms (nematodes) and flatworms (Platyhelminthes), flatworms are further divided into flukes (trematodes) and tapeworms (cestodes). The classification of helminths was originally based on their physical properties and the host organ they inhabit (Castro, 1996). Most worms that infect humans are so-called soil-transmitted helminths (STH). STH, commonly spread by eggs present in animal or human feces. The life cycle of all soil-transmitted helminths include eggs, larvae and adult worm stages. Typically, the eggs of STH are released into the soil following defecation and contamination of the soil by human feces. Depending on the species, the eggs either mature or hatch and become larvae. Larval growth and infective capacity depends on the environment; the time and the efficacy of larval development may vary substantially depending on humidity and temperature. Transmission of eggs to a new host most commonly occurs by the fecal-oral route; by ingestion of contaminated food or drinking water. Human hookworms are transmitted when infectious larvae get in contact with skin, which they penetrate. Following skin infection the larvae migrate through the blood and get via the lungs access to the intestines when mucus is swallowed. The adult worm develops in the intestines where male and female worms mate, the female produces new eggs, which hatch and develop into the third stage of larvae (L3) with infectious capacity to continue a new life cycle (Zaph et al., 2015).

1.1.2 Global Distribution and Epidemiology

Globally, more than 1.45 billion people, i.e. more than 20% of the world's population, are estimated to be infected with STH (Pullan et al., 2014). Human gastro-intestinal (GI)

helminthiasis are caused mainly by infection with *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale*. Approximately 1220 million are infected with *Ascaris*, 795 million with *Trichuris trichiura* and 740 million with the hookworms *Necator americanus* or *Ancylostoma duodenale*. Infections with more than one worm species in the same host are common (de Silva, N.R. et al., 2003).

Worm infections have become rare in industrialized countries, but remain wildly distributed in the less developed regions of the World, with the highest numbers of infections occurring in sub-Saharan Africa, the Americas, China and East Asia (de Silva, N.R. et al., 2003). In these regions, these infections affect the poorest and most deprived communities where sanitation is inadequate and water supplies unsafe (WHO 2012) (Figure 1).

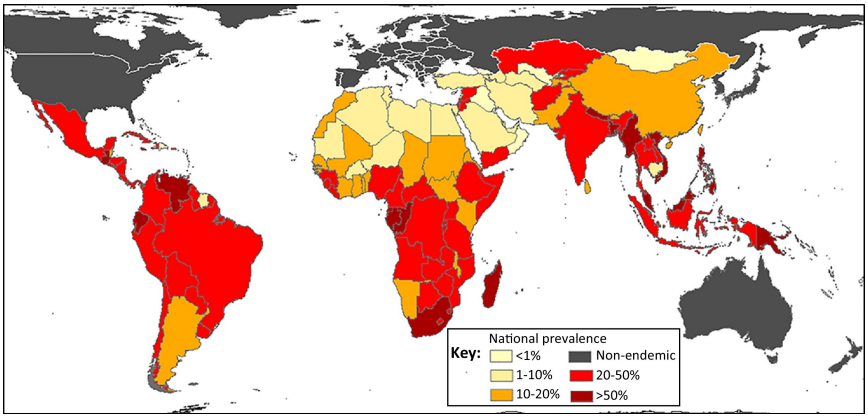

Figure 1. Global distribution of STH infection, 2010 (adopted from Campbell et al., 2016 *)

Helminth infections are common in all ages. However, children tend to be more affected and carry higher worm loads compared to adults. More than 267 million pre-school age children and over 568 million school-age children live in the areas where helminth parasites are intensively transmitted (WHO 2013). The intensity of infections with *Ascaria lumbricoides* and *Trichuris trichiuri* increase with age until reaching the peak around school age, to then decline in adolescence. Hookworm infections also increase with age in young children, with high prevalence and intensity in school-age children. Interestingly, for hookworms high prevalence can remain throughout adulthood (WHO 2012). This is a particular concern for pregnant women. Since these parasites suck blood, sometimes to the extent where the host may become anemic, they may affect the fetal development, causing neonatal prematurity, reduced neonatal birth weight, and increased maternal morbidity and mortality (Hotez et al., 2008).

1.1.3 Morbidity and Symptoms

While most of the 1.45 billion cases of STHs are relatively benign, worm infections can sometimes cause severe pathology and even be fatal. The symptoms of GI worm-infection can vary among individuals and include abdominal pain, ranging from mild to severe; anorexia; nausea; diarrhea; rectal prolapse; bowel obstruction, and allergies. Morbidity is directly related to worm-burden in the host. In fact, around 135 000 deaths every year are believed to be caused by high-burden infections with STH (World Health and Infecions, 2012). Importantly, STHs collectively cause substantial morbidity in millions of people. The chronic infections may result in malnourishment, impaired growth, delayed or impaired physical development and, in the case of hookworms, anemia (Anthony et al., 2007).

1.1.4 Diagnosis and Treatment of STHs

Intestinal helminths release eggs that are excreted in the feces, making stool the most convenient and widely used sample for detection of worm infection. Direct microscopy based on fecal egg counts remains the most used technique for diagnosis. The number of parasites in fecal specimens vary a lot and can sometimes be very low. This may require more advanced approaches for diagnosis. The technique named formal-ether concentration (FEC), first described by Ridley in 1956, can increase the concentration of eggs and is often applied to enhance the sensitivity of microscopic examination (Ridley and Hawgood 1956). Meanwhile, the Kato-Katz technique, which involves staining a sieved fecal sample and detecting eggs under a microscope is recommended by WHO for epidemiological surveys because of its simplicity and relatively low cost (Katz et al., 1972)**.** In consideration of the specificity, accuracy and reproducibility, several new techniques based on the egg-counting principle have been developed (Cringoli et al., 2010). The use of a McMaster microscopy chamber can increase the accuracy of egg counting (Vadlejch et al., 2011). Floatation based technique (FLOTAC), initially developed by Giuseppe et al., using the FLOTAC apparatus and based on the centrifugal flotation of a fecal sample suspension and subsequent analysis of the upper layer of the floating suspension, highly increased the light transmission and sensitivity of microscopic egg detection (Cringoli et al., 2010).

In the hands of well-trained technicians, microscopy allows both sensitive and species-specific detection. Molecular techniques such as PCR have also been developed to diagnose helminth infection. Unfortunately, these PCR-based methods are not more sensitive or specific than microscopy-based diagnosis but require more in terms of equipment and

reagents (Meurs et al., 2017). Methods based on antibodies or antigen for diagnosis of STH in stool samples are still not available for clinical use.

Although STHs can spread easily, they could nevertheless be controlled or eliminated by proper methods. The anti-helminthic drugs used today are well tolerated, are of low-cost and of high quality. The most commonly used medicines for treatment of GI nematode infections are broad-spectrum bensimidazole derivates such as albendazole and mebendazole. The mode of action for bensimidazoles is to block glucose uptake in the worm, which leads to energy depletion and impaired survival of the worm (Knopp et al., 2010). Other anti-helminthic compounds include levamisole and pyrantel. These drugs block acetylcholinesterase and reduce muscle contraction, paralyzing the worm and allowing it to be expulsed by the host (Whittaker et al., 2017).

Since reinfection with worms is common, whenever one individual in a household has been diagnosed, it is recommended that the entire household is treated to brake the transmission cycle (Bopda et al., 2016). In addition to treatment of the worm infection, it is crucial that sanitation is improved, that clean water is provided and that children are given good health education in the areas where worm infections are highly prevalent to reduce the risk of infection.

1.1.5 Re-infection and Drug Resistance

Treatment, while being highly effective and contributing to control of worm-infections rarely eliminate the parasites from the population. There are several reasons for this:

The first is because of post-treatment re-infection. Successful treatment does not lead to protection against subsequent infections. In fact, post-treatment re-infection is very common, with prevalence of *A. lumbricoides* and *T. trichiura* returning to almost pre-treatment levels within one year of drug administration if no additional measure to control spread of worms are implemented (Jia et al., 2012). Hookworm infections, on the other hand, tend to decline after treatment and re-infections are slower compared to other common soil-transmitted worm species. The high amount of egg production and long survival of the infective stage of *A. lumbricoides* in the environment may explain quick re-infection after treatment (Yap et al., 2013). The high risk of re-infection after therapy makes it difficult to control STHs and remains a challenge for its elimination. To control STH, long-term programs including community-based drug intervention and sanitary improvements are needed.

Moreover, resistance to anti-helminthic drugs is an increasing problem and may threaten our ability to treat these infections (James et al., 2009). This is of particular concern since there are few existing drugs available to treat worm infections. Development of drug resistance among parasitic nematodes is a risk that future health care systems need to take into consideration.

1.2 The Hygiene Hypothesis and Parasitic Worms

Although helminth infection remains a global and longstanding health problem, there is a concern that the complete elimination of helminths may cause new problems. Based on epidemiological data, the hygiene hypothesis, first proposed by David P. Strachan in the late 1980s, suggests that life style changes in industrialized countries have led to a decreased exposure to infectious agents and a consequent rise in allergic diseases (Sotgiu et al., 2008; Strachan, 1989). Support for this hypothesis has been gained through additional studies showing that children raised in countries or regions with high hygiene levels, are more likely to develop asthma compared to children that grow up in developing areas of the world.

Consistent with epidemiological findings, animal models have demonstrated a protective effect of worm infections against development of immunological disorders such as asthma, allergy and inflammatory bowel disease (IBD) (Evans and Mitre, 2015). Indeed, several studies indicate that helminthic infection may have positive effects on the host both by driving immune regulatory responses and by releasing molecules with immune modulatory capacity (Zaccone and Cooke, 2013). Immunomodulatory immune responses evoked by worms are discussed in more detail below. Furthermore, GI worm infections have been shown to protect the host from obesity and development of metabolic syndrome (Wong et al., 2007; Xiwei et al., 2015).

1.3 The Immune System

The immune system consists of lymphoid tissue, immune cells and their products (Janeway et al., 2001). The main function of the immune system is to prevent, control and eradicate infection. Lymphoid tissue is divided into central (or primary) lymphoid organs and peripheral (or secondary) lymphoid organs. In humans and other mammals, the bone marrow and thymus are the two generative lymphoid organs, responsible for lymphoid cell generation, proliferation and maturation. Peripheral lymphoid organs are lymph nodes (LNs), spleen, tonsils, mucosal-associated lymphoid tissue (MALT) and skin-associated lymphoid tissue (SALT). These secondary lymphoid organs offer a ‘room’ to optimize interactions

between antigen presenting cells (APCs), and lymphocytes so that the adaptive immunity can develop (Williams, 2012).

1.3.1 Generation of Lymphocytes

The bone marrow contains two main types of stem cells, mesenchymal and hematopoietic stem cells. The latter give rise to two main types of progenitors, the myeloid and the lymphoid cell lineages. Myeloid progenitor cells can give rise to monocytes, dendritic cells, macrophages, mast cells, neutrophils, eosinophils, basophils, erythrocytes and megakaryocytes. Lymphoid progenitor cells can differentiate into T lymphocytes, B lymphocytes and Natural killer T cells (NKTs) (Janeway et al., 2001) (Figure 2).

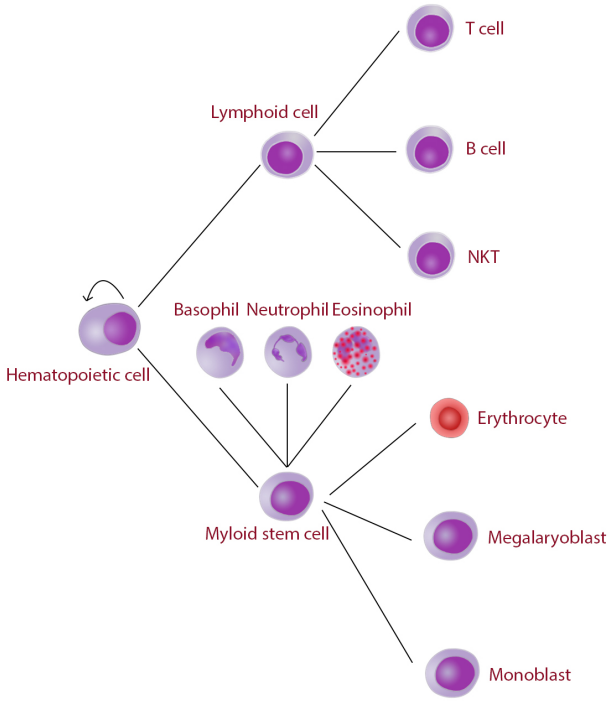

Figure 2. Hematopoietic cell differentiation

The maturation of lymphocytes from their progenitors consists of three steps: proliferation of immature cells, expression of antigen receptor, and selection of lymphocytes that express useful antigen receptors. T lymphocyte maturation takes place in the thymus while B lymphocytes mature in the bone marrow (Janeway et al., 2001). Firstly, IL-7 and other growth factors stimulate immature lymphocytes to proliferate, generating a large pool of immature lymphocytes. Secondly, expanded lymphocyte pools need to express a functional antigen receptor. The expression of antigen receptor on B and T lymphocytes is initiated by somatic recombination of gene segments. Lymphocyte progenitor cells from the bone

marrow inherently contain T cell receptor (TCR) and B cell receptor genes (Janeway et al., 2001).

In the third step, the maturation of T lymphocytes needs to undergo the selection by self-restricted MHC molecules. Cells with too low or too high self-affinity are selected against, preserving the ability to distinguish between self and foreign, while preventing dangerous self-reactivity. During this process, the T cell whose TCRs recognize class I MHC-peptide complexes maintain the CD8 expression while the TCRs that recognize class II MHC-peptide complexes preserve CD4 but lose CD8 expression (Janeway et al., 2001).

Once the T and B lymphocytes have completed their maturation, they can enter the lymphoid circulation. Until these cells meet and are activated by antigen, they are called naïve lymphocytes. The circulation of lymphocytes in the body takes place between blood, lymph and secondary lymphoid organs, including LNs, spleen, tonsils and Peyer's patches (Ganusov and Auerbach, 2014).

1.3.2 Lymphocyte Circulation

In humans, around 2 x 10^{12} lymphocytes make up the total lymphocyte pool. Most of these lymphocytes are continuously circulating in the body to monitor for invading pathogens (Lacey, 1982). Traveling through the blood, the lymphocytes reach peripheral lymphoid organs, where around 25% leave the blood stream and migrate into the secondary lymphoid organs. Naïve lymphocytes enter LNs by squeezing through "pockets" formed by the specialized high endothelial vessels (HEVs) (Mionnet et al., 2011). After random movements through the cortex and paracortex areas of the LN, lymphocytes accumulate in efferent lymphatic vessels, from which they can migrate into a nearby downstream LN or directly into large lymphatic vessels, eventually entering the main lymphatic vessel (thoracic duct), which empties into the blood (Young and Hay, 1995). Lymph flow in the human thoracic duct is about 1 mL/ min under homeostatic conditions (Ikomi et al., 2012). This continuous circulation of naïve lymphocytes is maintained unless they encounter an invading pathogen and are activated. The lymphocyte pool is maintained by a balance of cell input from primary lymphoid organs and cell death. Every day, about 2-2.3 million T lymphocytes are generated in the thymus to enter into the circulation and perform their functions or die if they have not been activated. Naïve T cells are estimated to have a life-span of around five to six months (den Braber et al., 2012; Campbell et al., 2003).

1.3.3 LN structure and Lymphocyte Movement in the LN

LNs are sentinel organs that monitor peripheral tissue for invading pathogens. LNs are bean-shaped and connected by lymphatic vessels. Afferent lymphatic vessels originate in distal tissue sites and drain into LNs. Lymph exits LNs by efferent lymphatic vessels, which can connect them to other LNs or empty back into the blood via the thoracic duct. LNs house stromal cells and large populations of lymphoid cells, mainly lymphocytes. Several types of stromal cells have been identified, such as lymphatic endothelial cells (LEC), follicular dendritic cells (FDCs), blood endothelial cells (BECs), and fibroblast reticular cells (FRCs) (Chang and Turley, 2015; Kedl and Tamburini, 2015). The majority of leukocytes in the LN are T lymphocytes and B lymphocytes. Natural killer cells (NKs), NKT cells, macrophages and dendritic cells (DCs) are found in lower frequencies. The LN is an encapsulated organ. It collects extracellular fluid containing cells and small molecules (lymph) via lymphatic vessels distributed in peripheral tissue. Under the capsule, there is a thin space called the subscapular sinus (SCS), which is connected with the lymphatic vessels. Macrophages lining along the SCS can capture antigens that enter via the lymph. B cell areas (follicles) and T cell areas (paracortex) are located under the SCS. These regions are separated by extended lymphatic vessels. HEVs, through which naïve lymphocytes enter the LN, are located in the border of the T and B cell areas and coil through the LN like branches of a tree. The medulla area is close to the paracortex and connects to the efferent lymphatic vessels where lymphocytes exit the LN (Mueller and Germain, 2009) (Figure 3).

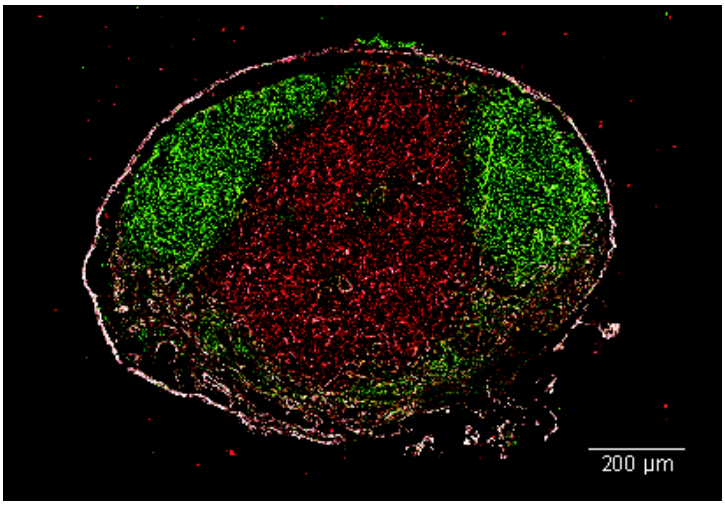

Figure 3. Section of pLN stained for CD3 (T cells) red and B220 (B cells green.

The outer capsule is outlined in white (Feng et al., 2018).

HEVs are composed of specialized vascular endothelial cells that express molecules crucial for lymphocyte entry. Under homeostasis, lymphocytes expressing CD62L (L-selectin)

initially attach to the peripheral node addressin (PNAd) expressed on HEVs. T lymphocytes can then roll along the vascular endothelium and become activated through the interaction of their surface receptor CCR7 with its ligands CCL19 and CCL21 on the vessel (Stein and Nombela-Arrieta, 2005). Activated lymphocytes then tightly bind to ICAM-1 on the endothelium via LFA-1 (CD11a), allowing them to enter into the LN parenchyma. T and B cells in the parenchyma slowly move into the paracortex and B cell-follicular areas, respectively (Figure 3). This migration is regulated by a gradient of the chemokines CCL19, CCL21, attracting T cells and CCL13, attracting B-lymphocytes. In the LN, lymphocytes "walk" along a network organized by FRCs or FDCs, scanning migratory DCs incoming from afferent lymphatic vessels and LN-resident DCs for antigen (Druzd et al., 2017). After some time, depending on the cell subset, lymphocytes accumulate into efferent lymphatic vessels and leave the LN (Tomura et al., 2008).

Using photo-activated "Kaede" transgenic mice, Tomura et al. found that different lymphocyte subsets migrate though the LN with a distinct movement and speed. In these mice, the replacement rate of LN lymphocytes ranged from 49% to 74%, per iLN per day. B cells had the lowest replacement rate and CD4 T cells the highest (Tomura et al., 2008). T lymphocytes move in an amoeboid-like manner at a speed of approximately 10-12 μm/min, while B cells move slower at about half the speed (Bousso and Robey, 2003; Qi et al., 2014; Tomura et al., 2008)

1.3.4 LN Remodeling after Infection

The LN microenvironment allows for naïve lymphocytes to recognize their cognate antigen presented on DCs, leading to the clonal expansion and differentiation of antigen-specific lymphocytes in response to an infection or antigen stimulation (Denton et al., 2014). During an infection, the expansion of the draining LN can be divided into four important steps. First, the afferent lymphatic vessels expand, enhancing the recruitment of antigen-presenting DCs from the periphery (Yang et al., 2014). Following this, the HEVs increase in size, number and permeability. The increased permeability of HEVs allows the entry of increased amounts of naïve lymphocytes (Mondor et al., 2016; Soderberg et al., 2005; Yang et al., 2014). Importantly, LN angiogenesis is initiated and sustained by factors derived from the inflamed peripheral site and the activated LNs itself (Tan et al., 2012). Third, the mediators secreted by cells belonging to the innate immune system, including TNF, type 1-interferon (IFN); contribute to the so-called LN shutdown. These cytokines stimulate naïve cells to up-regulate CD69, which causes down-regulation of the sphingosine-1-phosphate receptor (S1PR),

expressed on the lymphocytes. The down-regulation of S1PR prevents the lymphocytes to response to S1P, which is highly concentrated in the blood, causing lymphocytes retention. This step highly increases the chance for naïve lymphocytes to come in contact with DCs presenting their specific antigen (Schulz et al., 2014; Shiow et al., 2006). Lastly, when naïve lymphocytes have found their specific antigen presented on a DC, these lymphocytes are activated and proliferate dramatically (clonally expand), which further contributes to LN expansion (Min, 2018; Valitutti et al., 2010), discussed in more detail below.

1.3.5 Priming of Antigen-Specific Lymphocytes and T Cell Differentiation

Lymphocyte priming is defined as the process in which a naïve lymphocyte is activated to become an effector cell. The priming of antigen-specific T lymphocytes normally occurs during the first days after antigen stimulation or infection. This process of priming occurs through several continuous events, that can be divided into four steps: antigen recognition, activation, proliferation and differentiation (Janeway et al., 2001). As described above, naïve lymphocytes continuously migrate into the LN via HEVs. When arriving in the LN paracortex, they meet DCs presenting antigens. These DCs have migrated into the LN through afferent lymphatic vessels in a CCR7-dependent manner. The interaction between lymphocytes and DCs leads to the recognition of antigens presented on MHC by the specific TCR expressed on a T cell. At the same time, the lymphocyte receives additional signals from DCs for complete activation, CD4, or CD8 co-receptor recognize the MHC molecules, and the costimulatory molecules B7 on the antigen presenting DC can bind to the CD28 on lymphocytes, providing a second signal. In response to antigen and co-stimulation, activated antigen-specific T cells begin to proliferate, resulting in a dramatic expansion of antigen-specific T cell clones. Accompanying the proliferation, the progeny of antigen-stimulated proliferating T cells start to differentiate into effector cells capable of secreting effector cytokines that help combat the infection (Bajenoff and Guerder, 2003).

Effector T cells can be divided into helper and cytotoxic T cells, typically identified by their expressions of CD4 and CD8, respectively. Pending on the cytokine signals provided by the Antigen-presenting cell (APC), in most cases a DC, $CD4^+$ helper T cells differentiate into type-1 (Th1), type-2 (Th2), Th17 and regulatory (Treg) cells (Figure 4). The most important cytokine produced by Th1 cells is IFN-γ, which can stimulate the phagocytosis and killing of intracellular microbes. Th2 cells on the other hand, are hallmarked by interleukin (IL)-4 production, which can stimulate the production of IgE antibodies and IL-5, which in turn promote eosinophil activation. Th2 responses are central in immunity against helminths.

Th17 cells are characterized by secretion of the cytokines IL-17, IL-21 and IL-22 and are important for neutrophil recruitment and anti-fungal immunity. Moreover, in a microenvironment with retinoic acid and tumor growth factor (TGF)-β produced by DCs, naïve lymphocytes can differentiate to regulatory lymphocytes (inducible Tregs), which have the same function as natural regulatory T cells (Janeway et al., 2001).

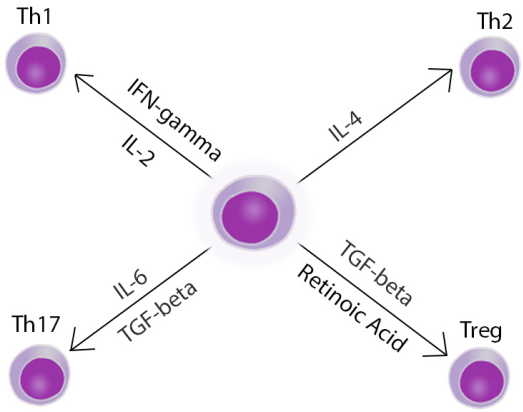

Figure 4. Differentiation of different $CD4^+$ T lymphocytes subsets

1.3.6 Migration of Effector T Lymphocytes to the Site of Infection

Following activation, differentiated effector T cells egress from the draining LN and migrate to the site of infection/inflammation. The entry of T cells into inflamed tissue is mediated by adhesion molecules on the T cells, which interact with ligands expressed on the endothelium. The recruitment of effector cells from the blood into an inflammatory site involves several steps. First, selectins (LFA-1) on effector cells interact with their carbohydrate ligands on blood endothelium cells, which enable the activated lymphocytes to firmly make contact with the vessel wall. Second, the chemokine IL-8, expressed on the blood endothelial surface, binds its receptor on the lymphocyte, enhancing the affinity. Third, LFA-1 binds to endothelial cell adhesion molecule ICAM-1, which results in the arrest of activated lymphocytes. Lastly, the activated lymphocyte changes its morphology allowing it to transmigrate across the endothelial barrier (Garrood et al., 2006).

1.3.7 Effector Function of Differentiated $CD4^+$ T Lymphocytes

Th1 lymphocytes activate macrophages, enabling them to phagocytize and kill ingested, intercellular microbes. The hallmark Th1 cytokine IFN-γ is excellent at stimulating macrophages. It triggers the transcription of genes that encode lysosomal proteases and enzymes. In turn, the expression of those enzymes can promote the synthesis of microbicidal

reactive oxygen species and nitric oxide (NO), which then mediate the killing of the intercellular pathogen. Th2 lymphocytes secrete IL-4 and stimulate IgE production by B cells (Janeway et al., 2001). Th2 responses are detailed in section 1.7.

1.4 Intestinal Immune Tissues

The intestine is the largest area of the body facing the external environmental. Structurally, the intestine comprises the small intestine (duodenum, jejunum and ileum), cecum, appendix and colon. Whereas the histology of the different segments of the small intestine slightly differ from one to the other, they are all built up in the same way. The intestinal wall is composed by four layers, the mucosal layer, the sub-mucosa, the muscularis and the serosa.

The immune cells and lymphoid structures of the GI tract are collectively referred to as MALT. Most immune cell populations are present in the intestine. DCs, macrophages and lymphocytes are distributed throughout the intestinal wall. Intraepithelial lymphocytes are believed to be part of a first barrier of defense against invading pathogens. In the lamina propria many different immune cells including activated T cells, plasma cells, memory lymphocytes, mast cells, DCs and macrophages can be found. Peyer's patches are another feature of the MALT, these are highly organized accumulations of immune cells that can act as a secondary lymphoid organ and with the mesenteric LN (mLN) promote the development of adaptive immunity (Ruddle and Akirav, 2010).

1.5 Mouse Strains Used to Understand Host – Parasite Interaction

Mouse models of helminth infection have contributed substantially to our understanding of T cell differentiation and control of infections. Different strain of mice commonly used in the laboratory show very different patterns in terms of resistance and susceptibility to helminth infections. For instance, infection of CBA or C57BL/6 mice with *H. polygyrus* results in a chronic infection, while BALB/c or SJL mice expulse the worms and are considered to be resistant (Filbey et al., 2014a). Genetic susceptibility and resistance were shown to be dependent on the immune response evoked in the different mouse strains, with the susceptible strains being more prone to generate Th1 responses, while resistant mice mounted strong Th2 responses. Similar immune response dichotomies have been reported in control of intestinal worm infections in humans (Ben-Smith et al., 2003).

1.6 Experimental Models of Worm Infection

In order to better understand host responses to GI helminths, several animal models have been used. These different models are believed to represent different aspects of human-

relevant intestinal helminth infection (Gouÿ De Bellocq et al., 2001; Reynolds et al., 2012). Much of the knowledge we have on immune responses to GI worms is based on these models, the most frequently used being *Heligmosomoides polygyrus, Nippostrongylus brasiliensis* and *Trichuris muris*. Even though these nematodes vary in their life cycle and route of transmission, they all spend the adult part of their life cycle in the GI tract, where they all induce type-2 immune responses. *H. polygyrus* is the worm model that we have used in our studies. We thus focus our observations on the use of this model.

1.6.1 *Heligmosomoides polygyrus*

H. polygyrus is a GI nematode frequently found in wild mice. It has been successfully adopted into the laboratory. Similar to other animal models of helminth infection, *H. polygyrus* offers a convenient model to explore the immunological processes during GI nematode infection (Maizels et al., 2012a, 2012b). Experimental infection is initiated by feeding the mice infectious L3 larvae. When reaching the small intestine, the larvae penetrate into the submucosa of the duodenum. This roughly occurs within 24 hours of infection. In the submucosa the larvae molt two times to become adults. The adult worms then migrate out of the submucosa into the intestinal lumen where they coil around the villi to survive peristaltic movements which can otherwise remove the worms. Mating of the male and female occurs in the lumen and generates eggs. Eggs are passed with the intestinal content and released in feces. Under favorable conditions (e.g. high humidity and mild to warm climate i.e. in a humid chamber at room temperature) the eggs hatch in soil after about 7 - 9 days and become infective L3. A new life cycle can then be initiated (Reynolds et al., 2012; Valanparambil et al., 2014).

H. polygyrus triggers a Th2 response in the early phase of the infection, detailed in section 1.7.2. Following this, the development of regulatory immune responses can be observed. This occurs after approximately 3 weeks post infection. Similar immunity and immunomodulation induced by nematodes can be observed in humans, indicating that *H. polygyrus* is a useful model to explore the mechanisms of immunity and immune evasion following GI nematode infection (Maizels and Smith, 2011).

1.6.2 *Nippostronglus brasiliensis*

N. brasiliensis is a natural parasite of rats in which the worm can cause a chronic infection. This worm can also infect mice. Compared to the other worm models, the life cycle of *N. brasiliensis* is more complicated and involves several host organs. The infection is initiated

when third-stage larvae infect the host by penetrating the skin. The larvae then migrate through the tissue and enter blood vessels within 6 hours post-infection. Flowing with the blood stream, these larvae access the lung parenchyma by bursting capillaries. The larvae grow and develop in the lung. After 1-3 days L4 larvae are released into the airways. The same host then swallows the larvae, due of the connection between airway and esophagus. The larvae undergoes a final molt, L5, and mature to adults in the intestine where they mate and produce eggs (Allen and Sutherland, 2014).

For *N. brasiliensis*, it has been shown that the protective immunity against infection is initiated when larvae are detected in the lung. Like most worms *N. brasiliensis* trigger strong type-2 immunity. In the gut IL-25 and IL-33 are produced by intestinal epithelial cells (IEC) early after infection, which is followed by the generation of Th2 cells. The clearance of this worm it is entirely attributed to the Th2-related cytokines IL-4, IL-5, IL-9 and IL-13.

The *N. brasiliensis* has been used to address lung stages of GI nematodes and to understand different wound healing mechanism, which are important to control the damage caused by the larvae. While this nematode share many similarities with human hookworms it has the disadvantage of only causing an acute infection in mice.

1.6.3 *Trichuris muris*

So far, more than 70 species of *Trichuris* have been recognized, most being species specific. *Trichuris muris*, a relatively common infection in wild mice, has been adapted to the laboratory. The *T. muris* life cycle is initiated by ingestion of eggs by a mouse, the 1st stage of larvae hatch in the caecum and penetrate the intestinal epithelial. In the intestinal wall, the larvae experience the first molt around 9-11 days followed by a second molt around 21 days post-infection. The third molt occurs at day 24-28 and the last molt takes place 29 days post-infection. After four molts the adult male and female worm are well developed and mate in the intestine. Afterwards, the young adults of *T. muris* can generate new eggs, which are released into the feces. Pending on the mouse strain and the dose of infection *T. muris* can cause an acute infection, which is clear or a chronic infestation, lasting for months. The *T. muris* model has often been used to study the difference in immune responses formed during the acute versus the chronic infection (Sorobetea et al., 2018). Resistance to *T. muris* is strongly related the establishment of type-2 immunity.

1.7 Immune Responses to Intestinal Worms

Our understanding, of the cellular and molecular mechanisms that mediate protective immunity to intestinal helminth infection is based on well-defined mouse models and data from human studies. Intestinal worms spend the greater and reproductive part of their life cycle in the intestinal tract. Accordingly, immune responses generated in the intestinal mucosa and the draining mLN are central for control of helminth infection.

1.7.1 Innate Immune Responses to Helminthic Infection

Compared to other parasites, worms are large. This prevents them from being ingested by phagocytes. In the gut worms are detected by small intestinal epithelial cells, which include tuft cells, mucus-secreting goblet cells, paneth cells and entero-endocrine cells. Signals from these cells are transmitted to the cells of the innate immune system. The main cytokines generated by epithelial cells in response to worms are IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) (Divekar and Kita, 2015). All of these cytokines strongly promote the expansion and proliferation of innate lymphoid cells type 2 (ILC2) in the lamina propria (Grencis and Worthington, 2016; Ji et al., 2016). ILC2s (and other cells) are activated to secrete IL-2, IL-4, IL-5, IL-9 and IL-13 (Filbey et al., 2014b; Pelly et al., 2016). IL-2 and IL-4 released from ILC2s are believed to be a source of cytokines for differentiation of Th2 cells following helminth infection (Ji et al., 2016; Pelly et al., 2016).

DCs are also central for induction of Th2 responses. While the exact role of DCs and DC subtypes is not fully known following helminth infection, it is clear that DCs are needed for development of Th2 responses. Phythian-Adams et al. demonstrated the importance of DCs during helminth infection by depleting $CD11c^+$. They found that the depletion of $CD11c^+$ DCs resulted in dramatically impaired Th2 cell differentiation and secretion of Th2-related cytokines (Phythian-Adams et al., 2010). Further, co-stimulatory signals from DCs are required for Th2 differentiation; blocking of CD80 and CD86 caused impairment of Th2 cell expansion and decreased IL-4 and IgE production (Lee and Iwasaki, 2007).

Macrophages can be divided into classically activated macrophages (CAMs or M1) and alternatively activated macrophage (AAMs or M2) according to their surface protein expression and function. Whereas CAMs are activated during Th1 responses, AAMs are generated and accumulated in infected tissue following infection with worms, including *H. polygyrus* and *N. brasiliensis* (Faz-López et al., 2016). The activation of AAMs is mediated by IL-4 and IL-13. AAMs are believed to play multiple roles during helminth infection. It has been suggested that AAMs indirectly play a role in Th2 development by downregulating the

Th1 response. Moreover and importantly, many helminths cause rather extensive tissue damage during their penetration and movement though host tissues. AAMs can contribute to wound healing by cleaning damaged matrix and cell debris (Mantovani et al., 2013).

In addition to the cells mentioned above, several other leukocytes have been described to having important roles in control of helminth infection. For example, granulocytes including eosinophils, basophils and mast cells may contribute to the expulsion of helminths from the host or impair their fecundity.

As mentioned above, worms cannot be ingested by single cells due to their large size. However, eggs and larvae (and sometimes adults) can physically be contained by granuloma structures restricting larval development and growth (Anthony et al., 2007a; Morimoto et al., 2004). In genetically resistant mice, more granulomas are observed around larva than during primary infection (Filbey et al., 2014b). These granulomas contain lots of cells, including $CD4^+$, $CD11c^+$ DCs, eosinophils, neutrophils and AAMs, the latter which appear to have an important function in control of re-infection (Anthony et al., 2007b).

1.7.2 Th2 Immune Responses to Intestinal Nematode Infection

Animals infected with worms typically generate a strong Th2 response, important for expulsion of the parasite and for resistance to re-infection (Pelly et al., 2016). Th2 cell development can first be seen as elevated gene expression of IL-4, IL-5, IL-9 and IL-13 in the mLN and Peyer's patches (Filbey et al., 2014b). By using IL-4-GFP reporter mice, studies of mice genetically defective in Th2 cytokines have shown that the IL-4/IL-4 receptor (IL-4R) signaling pathway is the most important effector response in control of *H. polygyrus*. Both IL-4 and IL-13 promote smooth muscle contractility in the upper intestine. This may explain how Th2 responses can facilitate worm expulsion (Zhao et al., 2003).

Experimentally, IL-4R signaling has been shown to be required for protective immunity to intestinal nematodes as such immunity is lost in the absence of IL-4R (Urban et al., 1991a). Importantly, both IL-4 and IL-13 utilize the IL-4R and these cytokines can have overlapping functions and can compensate for each other (Urban et al., 1991a).

The function of Th2 cytokines for the expulsion of helminths are different depending on worm species. IL-4 and IL-13 are as stated above, necessary for Th2 induction, smooth muscle contraction, B cell isotype switching from IgG to IgE production and worm expulsion.

The Th2-associated cytokine IL-5 and the cytokine IL-9 stimulate eosinophils and mast cells, respectively. Eosinophils are relatively abundant in the intestinal tract and can reduce the fecundity of nematodes like *H. polygyrus* (Behm and Ovington, 2000). However, while depletion of IL-5 significantly reduced eosinophil numbers it did not affect *H. polygyrus* expulsion (Urban et al., 1991b). IL-9 acts as a maturation factor for mucosal mast cells. Mast cell-deficient mice have been found to have an impaired capacity in clearing both *T. muris* and *H. polygyrus* (Faulkner et al., 1997; Forbes et al., 2008). IL-9 has also been suggested to contribute to smooth muscle contractility and intestinal peristalsis. Neutralization of IL-9 by antibody blocking lead to attenuated colonic muscle contractility and reduced expulsion of *Trichinella spiralis* (Khan et al., 2003). IL-9 has also been suggested to be important in expulsion of *N. brasiliensis* (Turner et al., 2013).

The Th2 cytokines IL-4 and IL-13 can stimulate and promote B cell activation and antibody isotype switching to IgE. Several studies have addressed the roles of B cells in response to intestinal helminth infection. The B-cell compartment is expanded and contributes to the enlargement of mLN during *H. polygyrus* infection (Wilson et al., 2010). IL-4-expressing T cells migrate to the T-B cell border, where they acquire signals for enhanced Th2 immunity (King and Mohrs, 2009). B cell-deficient mice showed an impairment of Th2 responses with decreased T cell expansion and reduced cytokine production (Wojciechowski et al., 2009). Moreover, expulsion of the nematode *T. spiralis* was found to be directly dependent on B cell responses, suggesting that antibodies produced by B cells bind to parasite surface and impair their migration. Additionally, IgE antibody produced by B cells can stimulate basophils to release vasoactive substances including histamine and cytokines IL-4, which also promote Th2 immunity.

1.7.3 Regulatory Immune Responses Triggered by Nematode Infection

The long-term survival of helminths in their host is the result of a process of dynamic co-evolution between the parasite and the host. The invading helminth had to evolve to enable its maturation and propagation without severely damaging or killing the host. A strong immune response can expel or even kill the parasite, but may also cause substantial tissue damage, which if not controlled can be fatal for the host (Motran et al., 2018). For this purpose, the worm modulates the immune system and suppresses both innate and adaptive inflammatory responses. Regulatory immune responses have evolved to reduce the harmful effects of an immune response. These regulatory responses, which serve to protect the host, also benefit the long-term survival of the worms. Indeed, many worms secrete molecules with immune regulatory properties (Finney et al., 2007; Johnston et al., 2017).

Tregs are characterized by their expression of the transcription factor Foxp3, high expression of the IL-2Rα chain (CD25) and production of the cytokines IL-10 and TGF-β. Finney et al. observed that following the establishment of Th2 responses during *H. polygyrus* infection, total $CD4^+CD25^+$ cell numbers and Foxp3 expression expand dramatically in the draining mLN (Finney et al., 2007). Consistent with this, infection with *H. polygyrus* in C57BL/6 mice lead to increased production of the regulatory cytokines IL-10 and TGF-β and expansion of $CD25^+Foxp3^+$ Treg cells in the mLN (Grainger et al., 2010). The regulatory response takes longer to develop than the Th2 response and in murine *H. polygyrus* infection they are found to be dominating around four weeks after infection. (Feng et al., 2018; Finney et al., 2007). Further, Rausch *et al*. demonstrated that Tregs can effectively suppress Th2 immunity to *H. polygyrus*, thereby facilitating persistent worm infection (Rausch et al., 2008). Moreover, a subtype of $CD11c^{low}$ $CD103^-$ DCs with a non-plasmacytoid origin has been shown to expand early after *H. polygyrus* infection. This DC subset preferentially induces Tregs, suggesting Treg modulation of immunity already early after infection (Smith et al., 2011). Additionally, *H. polygyrus* infection has also been shown to induce $CD8^+$ T cells with regulatory properties (CD8 Treg) in the lamina propria of the duodenum (Setiawan et al., 2007).

Analysis of the soluble secreted product from *H. polygyrus*, has identified a TGF-β mimic, which can act on the mammalian TGF-βR pathway and can enhance the expression Foxp3 in naïve peripheral $CD4^+$ T cells (Grainger et al., 2010; Johnston et al., 2017). This indicates that intestinal worms have developed an evolutionary mechanism to protect themselves by secreting molecules that dampen host effector immune responses (Rausch et al., 2008).

Recently, other immune modulating mechanisms by the parasite on the host has been found. Gillian et al. demonstrated that *H. polygyrus* releases extracellular vesicles (EVs), which are taken up by macrophages during the infection. The internalization of these EVs resulted in the suppression of both alternative and classical activation in macrophages (Coakley et al., 2017). Further, Buck et al demonstrated that *H. polygyrus* can secret vesicles containing microRNA. Theses microRNAs were protected by exosomes, and were internalized by the host cell where they could regulate innate immune responses by interfering with gene expression (Buck et al., 2014).

1.8 Helminth Infection and Immunity to Other Infections

As mentioned in section 1.2, data from human epidemiological studies and animal models indicate a relationship between helminth infection and increased susceptibility to other infectious diseases and a reduced risk of autoimmune disease.

1.8.1 Implications of Helminth Infection on Tuberculosis and Bacillus Calmette-Guérin (BCG) Vaccination

The immune regulatory effects of worms may also negatively influence other infections, in particular those that are controlled by Th1 responses, such as mycobacteria.

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB) remains one of the most wide-spread infections in the human population and is the top cause of death in the world due to do a single infectious agent. According to WHO around 10 million people developed TB and 1 million die because of this disease annually (WHO, 2017). The only vaccine against TB, Bacillus Calmette-Guérin (BCG), has been used around the World since the 1950's (Hawn et al., 2014). However, the efficacy of BCG varies among countries and populations and the reasons remain unclear. Of relevance to the work presented in this thesis, high prevalence of TB and low BCG efficacy geographically overlap with areas with high burden of intestinal helminth infections. Based on this association it was suggested that there may be a link between the development of TB, poor BCG vaccine efficacy and being infected with worms (Alemu and Mama, 2017; Elias et al., 2006).

Th2 responses, which hallmark helminth infection (Filbey et al., 2014a) can dampen Th1 responses, critical in turn for controlling mycobacteria and other intracellular infections of macrophages. Further, many worm infections drive Treg responses (Maizels and Smith, 2011). Tregs have inhibitory effects on all effector T cell responses, including Th1, Th2 and Th17. Thus, worm-driven expansion of Tregs may contribute to a weakened defense against other infections. In this respect, it has been shown that T-cell *in vitro* proliferation to secondary antigens was lower when the T cells originated from worm-infected compared to worm-free individuals. The reduced proliferative capacity was normalized when Treg cells were removed from these cultures (Wammes et al., 2010).

1.8.2 Impact of Worm Infection on Leishmaniasis

Leishmaniasis represents a major global health problem affecting more than 12 million people. The leishmaniases are a group of infectious diseases caused by parasitic protozoa of the genus *Leishmania*. The diseases can occur in various forms based on the nature of the

parasite species and the immune status of the host and are widely grouped into three types, classified by the infection site, namely cutaneous (CL), subcutaneous (or mucosal, MCL) and visceral leishmaniasis (VL). Cutaneous leishmaniasis is mainly caused by *Leishmania major, Leishmania aethiopica and Leishmania tropica* in Asia, Middle East, Europe and Africa. In the Americas *Leishmania mexicana* and *Leishmania amazonensis* are the main causes of CL. Specifically in South America the *Leishmania vianna braziliensis complex* contributes to CL, but infection with these parasites may, if not treated spread to the submucosa causing severe subcutaneous (MCL) disease. VL, which is the most severe form of leishmaniasis and almost always lethal if untreated, is caused by *Leishmania donovani and Leishmania infantum.*

Resistance and susceptibility to *Leishmania* has traditionally been associated with the dominance of Th1 or Th2. The adaptive immune response triggered by *Leishmania* is poised towards IFN-γ-producing Th1 cells in mouse strains that control the infection. In mice, activation of macrophages and NO production from macrophage contributes to the killing of *Leishmania* parasites. Mice strains like Balb/c which are biased towards Th2 responses and production of related cytokines IL-4 and IL-13, are highly susceptible to *L. major*, while C57BL/6 mice which are poised towards Th1 and inflammatory responses that control the infection (Gupta et al., 2014). However, this Th1-Th2 paradigm has not been able to predict susceptibility and resistance in humans. Subsequent studies have found that regulatory responses, in particular IL-10 is key cytokine for the failure to control *Leishmania* disease (Kane and Mosser, 2001; Nylén et al., 2007)

Since helminth infection promotes Th2 responses as well as regulatory responses and IL-10 production, a negative influence on the outcome of control of leishmaniasis could be envisaged. Studies on these co-infections are however scare. Newlove et al. showed that lesions in CL patients co-infected with helminths took longer time to heal when compared with lesions from patients without worms (Newlove et al., 2011).

2 OBJECTIVES OF THIS THESIS

The aim of this thesis was to investigate the effects of a chronic intestinal helminth infection on peripheral immune responses in the context of vaccination and co-infection.

Specific aims:

1. To determine if chronic intestinal nematode infection influences the outcome of secondary infection with Th1 controlled microorganisms.

2. To investigate mechanisms that can explain how intestinal nematode infection modulated immune responses to secondary infections or vaccination at distal sites.

3 METHODS

3.1 Maintenance of *H. polygyrus* Life Cycle and Preparation of Infectious L3 Larvae

H. polygyrus was originally a gift from Dr. H. Helmby (LSHTM, UK). To obtain infectious L3, feces containing eggs were collected from infected mice and mixed with activated charcoal (approximately 1:1 ratio) and distilled water to obtain a moist, non-runny paste. The mixture was the spread out on 2 layers' filter tissue in 10 cm diameter Petri-dish. The feces with egg-mix was kept in a humid chamber at room temperature. After 7-9 days, L3 larvae were detectable by microscope. To collect the larvae, the upper-layer tissue with above mixture was removed and the interface between the second filter tissue and dish was carefully rinsed. The distilled water containing larvae was collected into a 50 ml tube for washing. The tube containing larvae was left standing about 1 hour allowing the larvae to sink into the bottom. This washing step was repeated twice more or until the larvae was considered clean. After the final wash the L3 were diluted in 0.2% agarose and determined by microscopy, to obtain the desired concentration for infection (typically 200 L3/100 µl/mouse). The agarose solution was added to obtain and maintain a homogenous solution of L3, decreasing variability in infection load between animals.

For maintenance of the life cycle, infections were made in Swiss (CD1) mice. All experimental infections were performed in wild type (C57BL/6 or congenic Ly5.1/CD45.1) mice. If not otherwise mentioned, mice (4-5 weeks of age) were infected by oral gavage with 200 *H. polygyrus* L3 larvae, obtained as described above. Worm infections were considered chronic after 28 days. At the end of each experiment, the small intestine was collected, cut open and placed "inside-out" in a fine net placed in a 50 ml tube filled with medium (e.g. RPMI-1640 or DMEM). The intestines were placed at 37 ^{o}C to allow viable worms to migrate into the medium for 3-4 hours. Free worms were collected into a Petri-dish and counted by eye.

3.2 Secondary Infections

3.2.1 *Mycobacterium bovis* BCG

BCG strain SSI 1331 (Statens Serum Institute, Denmark) was expanded in 7H9 medium supplemented with ADC (Rothfuchs et al., 2009). For the immunization, 1×10^6 colony forming units (CFU)/30µl were inoculated in the footpad, ear pinnae (1×10^6 CFU/10µl) or injected by *i.v.* (1×10^6 CFU/100µl) via the tail vein as described elsewhere (Bollampalli et al., 2015). For quantification of mycobacterial load in tissue, single-cell suspension were

prepared and plated onto 7H11 agar supplemented with OADC, and incubated at 37^{o}C incubator for 21 days. The number of CFUs of mycobacteria were manually counted by eye.

3.2.2 *Leishmania major*

L. major, Freidlin (a gift from Dr. D. Sacks, NIAID, USA), was maintained in M199 supplemented with 20% FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 200 mM streptomycin. 1 x 10^5 metacyclic promastigotes in 10 µl of were injected into the ear dermis. For determination of *Leishmania* parasite burden, the ear was collected and homogenated. The suspension was cultured in serial dilutions in 96-wells plated with M199 medium supplemented with 20% FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 200 mM streptomycin in 37 ^{o}C for 7 days. Parasite load was determined using the last well of growth detected by microscopy and counting backwards according to the dilution factor(s) used (Sacks and Melby, 2001).

3.2.3 *Leishmania donovani*

L. donovani parasites were originally isolated in Bihar India (Srivastava et al., 2011). To generate large number of amastigotes, hamsters were infected. The spleens from *L. donovani*-infected hamsters were collected to purify amastigotes (Sacks and Melby, 2001). Amastigotes were stored at -150^{o}C until used for infection of mice. To infect mice, 1 x 10^5 amastigotes in 100 µl of DMEM were injected *i.v.* At the end of the experiment, to determine *L. donovani* load, DNA was extracted from liver and spleen tissues of infected mice. Parasite load was determined by qPCR using SsoAdvanced™ Universal SYBR® Green Supermix (BioRad) and *L. donovani* specific primers (Sadlova et al., 2013). The absolute number of *L. donovani* was obtained by comparing to a standard curve from titrated promastigotes of the same *L. donovani* strain cultured *in vitro*, as previously described (Sadlova et al., 2013).

3.3 Estimation of LN Cellularity and Blood Cell Counting

Superficial skin draining (popliteal, pLN; inguinal, iLN; and axillary, aLN) and total mesenteric LN (mLN) were collected in PBS at various time points following *H. polygyrus* and BCG infections. Cellularity was determined in single-cell suspensions of LN, prepared by crushing the LN with a pestle or to determine stromal cells following tissue digestion, as described by Broggi et al. (Broggi et al., 2014). Lymphocytes were counted by trypan blue exclusion, either by microscopy using a haemocytometer, or in an automated cell counter (Countess II, Life Technologies) or by FACS using counting beads (Countbright, Absolute bright count, Thermo Scientific). LN cell suspensions were >95% lymphocytes and

differences between counting methods were of negligible impact on results. For estimation of cells in blood, 20 µl venous blood was collected from the tail vein into EDTA-treated tubes at different times points following *H. polygyrus* infection. Cell counts were determined using an automated hematology analyzer (Mindray BC-2800Vet).

3.4 Phenotyping of Cell Subsets

3.4.1 Isolation of Stromal Cells from LNs

The iLNs were collected and kept in a sterile 5 ml polypropylene round-bottom tube containing 750-µl ice-cold basic mediums. 5 µl collagenase IV solution (1 mg/ml) and 3.5 µl DNase I solution (40 µg/ml) were added for primary digestion. One sterile magnetic stirrer was placed in each tube and the tubes were kept in the beaker with 37 ^{o}C pre-heated water. The mixture medium was stirred at a slow rate (1 round /second) for 30 min. Another 750 µl basic medium supplemented with 3.5 mg/ml collagenase D and 40 µg/ml DNase I was added into the tube. The mixture medium was stirred for another 5 min to further digest the tissue. LN tissue fragments were further disrupted physically by pipetting. Another 10 min digestion by the enzyme was performed and the reaction was stopped by adding 15 µl of 0.5 M EDTA (Broggi et al., 2014). Finally, the cell suspension was filtered through a 70 µm nylon mesh and centrifuged to collect the cells for further analysis.

For analysis of lymphocytes, LNs were only homogenized, washed and filtered before FACS staining and analysis.

3.4.2 Characterization of Lymphoid Cells and Stromal cells by FACS

FcγII/III receptor block was applied to avoid the unspecific antibody binding. Dead cells and debris were excluded by gating and use of LIVE/DEAD fixable dead cell staining kit, according to manufacturer's instruction (ThermoFisher, Cat.No.L34957). CD45 was used to distinguish between the lymphoid cells and stromal cell. In the CD45- stromal population, CD31 and gp38 were used to define the subset lymphatic vessel cells (LECs, $CD31^{+}gp38^{+}$), fibroblast reticular cell (FRCs, $CD31^{-}gp38^{+}$), blood endothelial cell including HEV cells (BECs, $CD31^{+}gp38^{-}$). In the $CD45^{+}$ population, MHCII I-A/I-E, CD11b and CD11c were using to gate the macrophages and dendritic cell. CD3, CD4, CD8 and CD19 were used to define $CD4^{+}$, $CD8^{+}$ T cell and B lymphocytes, respectively. Subsequently, CD62L and CD44 were used to define the naive ($CD62L^{hi}$ $CD44^{int/low}$), effector ($CD62L^{low}$ $CD44^{hi}$), central memory cell ($CD62L^{hi}$ $CD44^{hi}$) and effector memory cell. For $CD8^{+}$ cells, CD103 was used to confirm the naïve population ($CD103^{int}CD8^{+}$).

3.5 Cell Transfers and Tracking

P25-TCR Tg RAG$^{-/-}$ mice, with genetically modified Ag85B-specific T cell receptor (Tamura et al., 2004), designed to recognize the immune-dominant epitope of Ag85B present in both BCG and *M. tuberculosis,* were used to assess of Ag-specific T cells. Total LN from naïve P25-TCR Tg RAG$^{-/-}$ mice were collected and made into a single-cell suspension. 1 x 10^6 cells were injected into the recipient mice following BCG immunization. To detect transferred cells the LN were collected and lymphocytes suspensions stained with CD45.2 antibody and transferred cells were detected by gating on CD45.2^{+} cell by FACS.

To enable tracking distribution of different lymphocytes from naïve mice, lymphocyte were either collected from GFP-expressing mice or from unlabeled animals which were subsequently labeled with CFSE. The cells were then injected *i.v.* into the tail vein of recipient mice. Transferred cells were track in different secondary lymphoid tissues of recipient mice at various times points by FACS. Transferred cells were distinguished by GFP or CSFE expression and lymphocyte composition was determined as described above.

4 RESULTS AND DISCUSSION

Worm infections remain major global health problems in poor parts of the World with low sanitation (Novianty et al., 2018; Silver et al., 2018). Although STH infections rarely cause deadly disease, they collectively cause substantial morbidity. Importantly, it has been postulated that worm-infections may interfere with control of secondary infection and vaccination (Elias et al., 2006). However, results from studies on this subject have been diverging (Resende Co et al., 2007) and experimental proof to a large extent lacking. Results from a human BCG vaccine trial in worm-infected individuals indicated, that PPD responses following BCG vaccination, were impaired in worm-infected individuals (Elias et al., 2001). In the thesis presented here, we have investigated the effects of chronic worm infection on secondary pathogens and the immune responses evoked by the secondary infections. In particular, focus has been on how intestinal worms modulate immune responses distal to the site of worm infection.

Based on their findings in humans with and without worms Elias et al., suggested that intestinal nematodes would impair antigen-specific responses following BCG vaccination (Resende Co et al., 2007). Evidence was however based on a small number of individuals. As proof of principle, we aimed in **paper I**, to determine if the same could be demonstrated in an experimental model. The study by Elias et al. used a single dose albendazol to de-worm the study participants (Elias et al., 2006). This treatment primarily targets intestinal nematodes. We thus, selected a model of intestinal nematode infection that would be suitable to study intestinal worms. *H. polygyrus*, a nematode confined to the gut which cause limited pathology to the host (Maizels et al., 2012b), was deemed a good model for our purpose. We assessed how this nematode infection influenced immune activation and the outcome of secondary infections, both when the secondary infection was delivered systemically and when introduced in the skin, i.e. similar to how BCG vaccination is given in humans. To study antigens-specific responses we tracked transferred populations of mycobacteria Ag85B-specific $CD4^+$ T cells (P25-TCR-Tg). We found that P25-TCRTg responses to BCG were muted in mice with chronic but not acute *H. polygyrus* infection.

Purified Protein Derivative (PPD) is commonly applied as a screening test for TB or BCG vaccination and DTH skin text to PPD represents a tool to evaluate the specificity and functional status of memory cell-mediated immunity in humans (da Costa et al., 2011). To test the effect of worms on recall responses, we measured swelling in responses PPD injection in the footpad of mice. This mouse equivalent of the DTH test to PPD showed,

similar to observations acquired in humans, that the worm infection reduced recall responses to PPD (Elias et al., 2008).

In line with that immune responses were impaired, the infectious load of BCG following *i.v.* challenge was also higher in the livers of *H. polygyrus*-infected compared to worm-free mice. Similar to the result with mycobacteria, *H. polygyrus*-infected mice displayed an increase in parasite load upon secondary infection with *L. major* as well as a reduction in DTH responses to *Leishmania* antigens.

The immune modulatory effect of worms on mycobacterial responses has been ascribed to either regulatory responses, Th2 responses or both. A systemic spread of worm-induced Th2 and/or regulatory cytokines or cells could explain how local this intestinal infection influenced immunity to a co-infection (Mohrs et al., 2005). Indeed, we observed a reduced expression of genes associated with mycobacterial control (iNOS, IFNγ, TNF) in livers of *H. polygyrus*-infected mice compared to worm-free mice upon BCG challenge. However and in contrast to reports from others (Liu et al., 2009; Mohrs et al., 2005), we found no support for dissemination of Tregs (FoxP3) or Th2 cells in mice with chronic *H. polygyrus* infection.

Many parasitic worms can secrete molecules with immune modulatory effects (Maizels et al., 2012a; Motran et al., 2018). *H. polygyrus* generates excretory secretory molecules (HES) with TGF-β signaling capacity (Johnston et al., 2017). TGF-β is a pluripotent cytokine, which in the immune system has inhibitory activities on inflammatory responses. The HES product signalling through the TGF-βR was identified a structurally distinct TGF-β mimic (TGM), which can induce Tregs via TGF-βR (Johnston et al., 2017). *In vitro* we found that HES act on T cells via the TGF-βR and inhibit mycobacteria-specific T-cell priming, indicating that TGF-β, worm- or host-derived, could play a role in worm-induced inhibition of secondary immune responses and promoted its function to be characterized *in vivo*. It has been reported that mice infected by *H. polygyrus* increase TGF-β serum levels (Donskow-Łysoniewska et al., 2012). Our data indicated that chronic *H. polygyrus* infection might increase production of TGF-β, since more latency-associated peptide (LAP) was detected in that splenic $CD4^+$ cells from mice with worms compared to worm-free mice.

However, while the Foxp3 expression was increased both in number and percentage in the mLN which drains the intestinal worm infection, no difference was observed in distal sites (e.g. pLN) compared to *H. polygyrus*-free mice, indicating that the impaired immune

response is not attributed to the systemically dissemination of regulatory immune response (Paper 2).

We observed that this worm infection, localized to the gut impaired BCG-specific $CD4^+$ T cell priming in both spleen and skin-draining LN and decreases their cytokine expression, which supports the view that intestinal worms diminish the immune response to second infection or vaccinations (Elias et al., 2008).

DCs are phenotypically diverse cells that play a fundamental role in the sensing of pathogens, transport and presentation of antigens. Migration of DCs from the site of infection to the draining LN, where T cells are primed is a key event in the initiation of an immune response (Alvarez et al., 2008; Colonna et al., 2006). Since T-cell priming, in responses to BCG, was diminished in worm-infected animals the impact of worms and worm-derived molecules was of relevance to investigate further. In this regard, we tracked DC migration from the site of injection to the draining LN in mice with or without intestinal worm infection. To do this we developed a method of CFSE into the skin of footpad to follow DCs migrated from skin to pLN after BCG injection (Bollampalli et al., 2015) The majority of migrating cells ($CFSE^+$) were MHC-$II^{hi}CD11c^{+/int}$, indicating the phenotype of migratory DCs. When we compared mice with and without chronic *H. polygyrus* infection, we found fewer $CFSE^+$ MHC-$II^{hi}CD11c^{+/int}$ in pLN compare to *H. polygyrus*-free mice, indicating that DC migration in response to an antigen delivered in the skin was influenced by intestinal nematodes.

Taken together, we experimentally demonstrated that an intestinal helminth can mute T-cell responses to mycobacteria and impair control of secondary infection/vaccination distal to the gut. Some of our data pointed to that TGF-β could have a role in down-regulating immune responses in worm-infected animals, however the underlying mechanism of the impaired immune response could not be attributed to the systemic dissemination of Th2 or Tregs.

In **paper II** we continued to investigate the mechanism underlying the impaired immune response to secondary infection/vaccination in animals with intestinal worm infection. We observed that, the number of migratory DCs and antigen-specific cells upon BCG vaccination was highly correlated to the cellularity of the draining LN. This prompted us to further investigate how the worm infection on its own, influenced LN cellularity. Indeed, we observed a reduced cellularity in peripheral LN of worm-infected compared to worm-free mice, which is in line with data by King *et al*. (King et al., 2017), indicating that the worm, by modifying the host immune homeostasis, influences the immunity to a second

antigen/pathogen. Development of this cellular LN atrophy was time-dependent and only significant after 3-4 week of *H. polygyrus* infection. The reduction of cellularity in LN distal to the infectious site was accompanied by alteration of the cellular composition, with T cells being lost over B cells, while the stromal cell population (LECs, BECs, DNCs and FRCs) and DCs and macrophages, were not significantly altered. The worm infection did not appear to affect body growth or cause any severe disease in the mice, which could explain the LN atrophy. Worm-infected mice had a healthy appearance and gained weight similar to worm-free mice.

To better understand how intestinal worms affected distal LNs we assessed gene expression in inguinal LNs from worm-infected and worm-free mice. In line with observations made in co-infected mice, RNA-sequencing data did not point to an accumulation of Th2 or Tregs in the peripheral LN from *H. polygyrus* infected-mice compared to *H. polygyrus*-free mice, suggesting that other factors may be more important in affecting LN cellularity. We also speculated that cell death, which would not necessarily manifest in gene expression assays, could be an explanation to the reduced LN cellularity in worm-infected mice. However, we found no support for the loss of lymphocytes in the skin-draining LN of *H. polygyrus*-infected mice to be due to worm-induced apoptosis (Cliffe et al., 2007). This indicates that other underlying mechanisms must mediate LN atrophy.

T and B lymphocytes are generated in the thymus and bone marrow respectively, which contribute to the filling of the lymphocyte pool, including the circulating lymphocytes in blood and lymphatic vessels and the cells in lymphoid tissue (Andrade et al., 1998). The maintenance of the T lymphocyte pool requires daily output from thymus and equal amount of programmed cell death during homeostasis (Tanchot and Rocha, 1998) . Infections, as well as inflammatory diseases, have been found to influence thymic output (De Meis et al., 2012). Our analysis of *H. polygyrus*-infected animals did not indicate that the thymic output was affected by intestinal *H. polygyrus* infection. Thus, we sought other explanation to the peripheral LN atrophy caused by intestinal nematode infection.

The homeostatic maintenance of LN volume requires the influx of lymphocytes from afferent lymphatic vessels and HEVs respectively, with equal amount of leukocytes exiting through the efferent lymphatic vessels (Mionnet et al., 2011). Upon infections, the rapid expansion of the draining LN increases the chance of encounter between antigen-presenting DCs and naïve, mature antigen-specific lymphocytes. Soderberg et al. has showed that large amount of naïve lymphocytes accumulate in the draining LN upon viral infection (Soderberg et al.,

2005). Consistent with this, the size and the number of naïve lymphocytes increases dramatically upon *H. polygyrus* infection. This expansion of mLN continues until the infection is chronic, when the mLN cellularity stabilizes.

Additionally, the reduction in peripheral LN cellularity was parallel by an increase in cellularity in the mLN. We thus hypothesized that the massive recruitment of lymphocytes to the mLN could alter the balance of the distribution of the lymphocyte pool throughout the system. By using different approaches to track transferred lymphocyte populations we found that the mLN of *H. polygyrus*-infected mice retained more transferred lymphocytes compared to *H. polygyrus*-free mice while the reversed was the case for the iLN. To confirm that the mLN expansion included the recruitment of naïve lymphocytes and not caused by cross reactivity of transferred cells, we injected mycobacteria Ag85B-specific P25-TCRTg cells into *H. polygyrus*-infected and *H. polygyrus*-free mice. These cells which cannot recognize worm antigen were also found in higher numbers in mLN and in lower numbers in iLN of worm-infected compared to worm-free mice, supporting our hypothesis that redistribution of lymphocytes induced by nematode infection modulate the immune system.

Taken together, our data indicated that muted peripheral immune responses to BCG infection in worm-infected animals are best explained by redistribution of lymphocytes and atrophic skin draining LN. De-worming has been proposed as a measure to improve vaccine efficacy and removal of worms have been found to increase immune responses to BCG and malaria parasites when compared to individuals still harboring worms (Elias et al., 2001; Wammes et al., 2016). We thus tested if anti-helminthic treatment would lead to the recovery of the skin-draining LN rescue the immune response generated in response to a secondary co-infection. Our data suggest, that although mLN remained enlarged even three weeks after de-worming treatment, the cellularity of skin-draining LNs recovered and was almost equal to that of worm-free mice. Further, upon BCG injection, the number IFN-γ-expressing $CD4^+$ T cell found in the BCG draining LN were comparable in *H. polygyrus*-infected and de-wormed and control mice (never infected with worms). This indicates that de-worming, and time given for the LN to recover cellularity, can improve the immune response to vaccines given in the periphery.

In **paper III**, we showed using yet another model of secondary infection, namely *L. donovani*, the causative agent of VL, that worms also negatively affect the outcome of this infection. Similar to the observations made using BCG we found that *L. donovani* parasites survived better in the worm-infected compared to the worm-free mice and that the correlation

between Th1 cytokine expression and worm control was lost in co-infected animals (Obieglo et al., 2016).

Taken together, we have made a comprehensive analysis of how chronic intestinal worm infection can influence the outcome of secondary infections, using mouse models of co-infection. Systemic and distal infections as well as immune responses were affected by the worm localized in the gut. While dissemination of worm induced Th2 and regulatory response cannot be excluded in the spleen and liver, redistribution and competition for lymphocytes better explain why hosts chronically infected with worms mount weaker responses to vaccination and secondary infections compared to people who are worm-free or have been de-wormed (Mishra et al., 2014).

5 CONCLUDING REMARKS

Geographically, high prevalence of STHs overlaps with low BCG vaccination efficacy and high prevalence of TB. This has led several groups to suggest that intestinal nematodes may impair antigen-specific responses following BCG vaccination (Elias et al., 2006; Resende Co et al., 2007).

We provide experimental evidence that an intestinal nematode infection influences immune activation and the outcome of secondary infection both systemically and locally in the skin. Similar to observations made in humans, recall immune responses were also affected by worm infection, indicating that worms may also hamper diagnosis of TB.

We tried to explain these observations by dissemination of Tregs induced but found no solid support for dissemination or increase in regulatory immune responses in the peripheral skin-draining LNs.

The strength of an immune response is balanced by effector and regulatory responses, but also dependent on the number of immune cells available to respond to a specific pathogen. We found that the peripheral LN becomes atrophic given enough time following the worm infection. The lymphocyte loss is primarily attributed to the recruitment of naïve lymphocytes into the mLN, draining the site of worm infection. The accumulation of naïve lymphocytes into the draining LN cause a redistribution of the lymphocyte pool throughout the body, which results in a weaker immune response to a second infection introduced at a distal site. De-worming, lead with time lead to a recovery of cellularity in skin-draining LNs.

Taken together, we have found that immune cell distribution is altered during chronic worm infection. The lack of available lymphocytes in skin-draining LNs leads to the impaired immune response to a subsequent infection given in the skin.

6 ACKNOWLEDGEMENTS

The project presented here was supported and financed by research funding from: **Åke Wibergs Stiftelse**, **Vatenskapsrådet**, **Karolinska Institutets fonder**, **Claes Groschinskys Stiftelse**. This work is also supported **by Chinese Scholarship Council**.

I have spent 7-month of pre-PhD study followed by 4 years and 3.5 months of PhD work in Karolinska Institutet, during which I have received lots of help and support from friends and colleagues:

First and foremost, I would like to thank my supervisor, **Susanne Nylén**, for recruiting me into your lab and guiding me through this fantastic project. I really appreciate that you have paid attention to and, at most of the time, tolerated my poor English expression. The immunology study group that you organized has also highly strengthened my basic knowledge. Your suggestion that 'You should present your project to a person who is in a complete different area' has always prompted me to do the research in a substantial way. You also taught me the importance of enjoying life. Thanks for every experiences and moments you have given me, I definitely will not reach where I am now without your support during the past years.

Mats Wahlgren, my co-supervisor, you are such a nice person. Thanks a lot for creating the free-style work environment in science and I really enjoy it. I still remember that you said: do more when you enjoy it and relax when you feel exhausted. You have also once told me 'Don't always believe in the authority, try to argue and explore.' I will keep it in mind and do my best in the future.

Martin Rottenberg, my co-supervisor, thanks for recommending me to Susanne's group and also thanks for hosting the journal clubs which have always enlightened me with new ideas and knowledge on how to design experiment to address a scientific problem.

Antonio Gigliotti Rothfuchs (**Tony**), my co-supervisor, I appreciate your support in my project and give so much help in our paper work. Thanks for your patience during our talks and preparation of the experimental protocols. Also, thanks for keeping me on track of my project all the time.

Anders Björkman, Thank you for being a great mentor and for your greeting during my study here.

Jonathan M Coquet, I would like to say a big thanks to you. Though you may not be my co-supervisor on the paper, but you have undoubtedly assumed a role as a co-supervisor to me during the past years. I couldn't forget that you spend the whole morning showing me how to calculate the cellularity of LN, which has inspired me to try to address science questions in a flexible, but logical, way. Of course you always push me to do the work step by step. I learnt it and enjoy consulting you whenever I have new ideas. The thesis work will definitely take much longer to complete, should your help be not available.

Cajsa Classon, you are the only but best colleague in this project. It is so nice to work with you for the animal work and the preparation of the manuscript. Working with you always makes me feel relaxed and fully supported. With you being here, I felt extra strength in myself to solve problems in the lab.

Sherwin Chan, I am so lucky to have you as a senior in our big lab. Thanks very much for paying attention to my hypothesis and giving me advice on how to refine it, especially that you are working in completely different areas of interest. Also thanks for your assistances on many technical issues in the lab. Most importantly, thanks for your advices in life and your patience in listening to my whining all the time.

Yunlong Yang, you are all-round supportive to me. Thanks for the scientific discussions and advices on my work. The work on blood concentration kinetics will not be possibly done without your generous help. Thanks for the suggestions on how to deal with problems in work and in life.

Jiyu guan & Fei Gao, Thank you for encouraging me to pursuit a PhD study in Karolinska Institutet. Thanks for giving me lots of advices in the life when I first arrived in Sweden. Thanks for the 'chit-chat' that bears no boundary.

Ulf Ribacke, I enjoy so much of your 'silly' jokes in the office. It is much more relaxing to work in this harmonious atmosphere created by you. Thank you for teaching me the techniques important for the project. You share so much funny story and give very meaningful suggestions on my future career.

Åsa Sjöling, thanks for being the chairperson on my yearly-follow up and half time control. Thanks for your patience and questions during my presentation.

Lisa Westerberg, thanks for being the examiner during my half time control. It was a very stimulating discussion and I learned a great deal from it.

Kenth Andersson, the 'king' of animal facility, your superb techniques and dexterity on sample collection have certainly saved many of my days from the need to work till very late. Thanks for all the jokes but also giving essential guidance for the animal work.

Velmurugesan Arulampalam, Thanks so much for the support during my PhD study and your unmeasurable kindness and patience given to me. **Åsa Belin,** Thanks very much for your kind help on all necessary paper works during my PhD study.

Jasmina Bier, it was an honor to guide you as a summer student. I taught you technique but also learned a lot from your strong spirit on science. Thanks for trusting me.

I would also like to show my gratitude to all the colleagues in the big corridor during my PhD study. **Martina Löfstedt Jalava**, thanks for ordering chemicals, reagents and dealing with the invoices. I appreciated your patience both during work and lunchtime. **Pilar Quintana Varon**, Thanks so much for sharing your experience in the office and lab, Thanks for your support and help during my PhD study. **Daisy Hjelmqvist**, it is so nice to be registered with you on the same day and start our PhD journey, thanks for the help on everything and your patience during our talks. I wish you good luck in your own PhD journey. **Chim Chan**, I still remember all the talks between us and the tips you gave to me on how to further explore my study. **Pablo Giusti**, technically advanced FACS master, thanks for teaching me all the skills on FACSing. **Caroline Rönnberg**, you are such a responsible doctor and teacher, I learned a lot from you during the parasitology teaching course. **Akira Kaneko**, you are a very admirable scientist and generous person, thanks for all the talks and invitations. I would also like to show my appreciations to the others: **Anna Bereza-Jarocinska**, **Manual Patarroyo, Zulkarnian MD Idris, Junhong Ch'ng, Berit Schmidt, Kirsten Moll**, **Caterina Scasso**, **Ulrika Morris**, **Irina Jovel-Dalmau**, **Mubasher Mohammed, Alejandra Frasch**, **Viktor Persson**, **Pontus Hedberg**, **Madle Sirel**, **Hannah Akuffo**, **Juliana Inoue**, **Maria Pena Fransch**, **John Nyberg**, **Atiqul Islam**, **Rory Barnes** and **Ernest Moles**. Thanks for sharing with me many enjoyable moments in the lab, I will remember every bits and pieces in the years to come.

I would also like to thank all colleagues in the journal club: **Jintao Guo**, **Graciela Téran**, **Limei Wang**, **Vishnu Priya Bollampalli, Berit Olsson, Berit Carrow, Damien**

Bierschenk, Juan Basile, Nuno Ramos Rufino de Sousa, Veronika Krmeska, Lei Shen, Ruining Liu, Beston Hamasur, Malin Eriksson and Leonie Vetter.

Yu Gao, Thanks for the help during the early time when I first arrived in Sweden. Also thanks for the support during my study and discussions concerning work and life in Sweden.

Lastly, I would also like to show my appreciations to: **Kayoko Hosaka**, **Yonas Bekele**, **Christ Tibbitt**, **Julian Stark**, **Eduardo Villablanca**, **Chiara Sorini**, **Toruun Söderberg**, **Per Person**, **John Sennett**, **Hans Wigzell, Stanley Cheuk.**

The friendship with all of you during my time in Sweden: **Fan Zhang** & **Fan Yang**, **Dongmei Tong** & **Tenghao Zheng**, **Zhe Zhang** & **Shengze Yu**, **Fengyang Li**, **Sichao Li**, **Junwei Zhang**, **Min Guo**, **Honglei Zhao**, **Lifeng Liu, Jingya Yu**, **Limin Ma** & **Björn Koop, Ying Wang** & **Yu Zhao, Mo Zheng & Xiaokuan Li, Weiping Wu & Jianqiang Xv, Hanyan Ye& Adam Köhegyi, Xiaoyun Chen, Yunyu Wang, Lin Shi**.

奶奶，谢谢您对我的教育和悉心的照顾以及无条件的支持。**爸&妈**，谢谢您们对我的养育之恩以及对我学业生活的大力支持和关心。**哥&嫂，弟**：谢谢你们在我留学期间对家人的照顾，能够使我安心完成学业，谢谢你们。**冯梓纯**，亲爱的小侄女，祝你健康成长，开开心心。同时也感谢**磊爸**，**磊妈**，**王法**姐夫，**李冰**姐和**王歆然**小同学对我学业的鼓励和支持 。

李磊，我未来一生的亲密战友。谢谢你鼓励我趁早出国求学，并给与我的大力支持。感谢你对我生活无微不至的关心和照顾; 感谢你对我工作中的各种支持; 感谢你让我对未来充满信心, 感谢一路有你。我爱你，一辈子!

Thanks Karolinska Institutet, Stockholm, Sweden!

7 REFERENCES

Alemu, G., and Mama, M. (2017). Intestinal helminth co-infection and associated factors among tuberculosis patients in Arba Minch, Ethiopia. BMC Infect. Dis. *17*, 1–9.

Allen, J.E., and Sutherland, T.E. (2014). Host protective roles of type 2 immunity: Parasite killing and tissue repair, flip sides of the same coin. Semin. Immunol. *26*, 329–340.

Alvarez, D., Vollmann, E.H., and von Andrian, U.H. (2008). Mechanisms and Consequences of Dendritic Cell Migration. Immunity *29*, 325–342.

Andrade, W.N., Johnston, M.G., and Hay, J.B. (1998). The relationship of blood lymphocytes to the recirculating lymphocyte pool. Blood *91*, 1653–1661.

Anthony, R.M., Rutitzky, L.I., Urban Jr., J.F., Stadecker, M.J., Gause, W.C., Urban, J.F., Stadecker, M.J., and Gause, W.C. (2007a). Protective immune mechanisms in helminth infection. Nat. Rev. Immunology *7*, 975–987.

Bajenoff, M., and Guerder, S. (2003). Homing to Nonlymphoid Tissues Is Not Necessary for Effector Th1 Cell Differentiation. J. Immunol. *171*, 6355–6362.

Behm, C.A., and Ovington, K.S. (2000). The role of eosinophils in parasitic helminth infections: Insights from genetically modified mice. Parasitol. Today *16*, 202–209.

Bellagio, R.F. (2017). Reaching girls and women of reproductive age with deworming.

Ben-Smith, A., Lammas, D.A., and Behnke, J.M. (2003). The relative involvement of Th1 and Th2 associated immune responses in the expulsion of a primary infection of Heligmosomoides polygyrus in mice of differing response phenotype. J. Helminthol. *77*, 133–146.

Bollampalli, V.P., Harumi Yamashiro, L., Feng, X., Bierschenk, D., Gao, Y., Blom, H., Henriques-Normark, B., Nylén, S., and Rothfuchs, A.G. (2015). BCG Skin Infection Triggers IL-1R-MyD88-Dependent Migration of EpCAMlow CD11bhigh Skin Dendritic cells to Draining Lymph Node During CD4+ T-Cell Priming. PLoS Pathog. 2015 Oct 6;11(10):e1005206.

Bopda, J., Nana-Djeunga, H., Tenaguem, J., Kamtchum-Tatuene, J., Gounoue-Kamkumo, R., Assob-Nguedia, C., and Kamgno, J. (2016). Prevalence and intensity of human soil transmitted helminth infections in the Akonolinga health district (Centre Region, Cameroon): Are adult hosts contributing in the persistence of the transmission? Parasite Epidemiol. Control *1*, 199–204.

Bousso, P., and Robey, E. (2003). Dynamics of CD8+ T cell priming by dendritic cells in intact lymph nodes. Nat. Immunol. *4*, 579–585.

den Braber, I., Mugwagwa, T., Vrisekoop, N., Westera, L., Mögling, R., Bregje de Boer, A., Willems, N., Schrijver, E.H.R., Spierenburg, G., Gaiser, K., et al. (2012). Maintenance of Peripheral Naive T Cells Is Sustained by Thymus Output in Mice but Not Humans. Immunity *36*, 288–297.

Broggi, M. a S., Schmaler, M., Lagarde, N., and Rossi, S.W. (2014). Isolation of murine lymph node stromal cells. J. Vis. Exp. 2014 Aug 19;(90):e51803.

Buck, A.H., Coakley, G., Simbari, F., McSorley, H.J., Quintana, J.F., Le Bihan, T., Kumar, S., Abreu-Goodger, C., Lear, M., Harcus, Y., et al. (2014). Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. Nat. Commun. *5*, 1–11.

Campbell, D.J., Kim, C.H., and Butcher, E.C. (2003). Chemokines in the systemic organization of immunity. Immunol. Rev. *195*, 58–71.

Campbell, S.J., Nery, S. V., McCarthy, J.S., Gray, D.J., Soares Magalhães, R.J., and Clements, A.C.A. (2016). A Critical Appraisal of Control Strategies for Soil-Transmitted Helminths. Trends Parasitol. *32*, 97–107.

Castro, G.A. (1996). Chapter 86 Helminths : Structure , Classification , Growth , and Development Flukes (Trematodes). 1–6.

Chang, J.E., and Turley, S.J. (2015). Stromal infrastructure of the lymph node and coordination of immunity. Trends Immunol. *36*, 30–39.

Cliffe, L.J., Potten, C.S., Booth, C.E., and Grencis, R.K. (2007). An increase in epithelial cell apoptosis is associated with chronic intestinal nematode infection. Infect. Immun. *75*, 1556–1564.

Coakley, G., McCaskill, J.L., Borger, J.G., Simbari, F., Robertson, E., Millar, M., Harcus, Y., McSorley, H.J., Maizels, R.M., and Buck, A.H. (2017). Extracellular Vesicles from a Helminth Parasite Suppress Macrophage Activation and Constitute an Effective Vaccine for Protective Immunity. Cell Rep. *19*, 1545–1557.

Colonna, M., Pulendran, B., and Iwasaki, A. (2006). Dendritic cells at the host-pathogen interface. Nat. Publ. Gr. *7*, 117–120.

da Costa, N.M.X., de Albuquerque, M., Lins, J.B.A., Alvares, J.T., and Stefani, M.M. de A. (2011). Resposta de testes de hipersensibilidade tardia utilizando PPD e outros antígenos em crianças e adolescentes saudáveis e infectados pelo HIV-1 e vacinados com BCG. Rev. Soc. Bras. Med. Trop. *44*, 542–545.

Cringoli, G., Rinaldi, L., Maurelli, M.P., and Utzinger, J. (2010). FLOTAC: New multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat. Protoc. *5*, 503–515.

Denton, A.E., Roberts, E.W., Linterman, M.A., and Fearon, D.T. (2014). Fibroblastic reticular cells of the lymph node are required for retention of resting but not activated CD8+ T cells. Proc Natl Acad Sci U S A *111*, 12139–12144.

Divekar, R., and Kita, H. (2015). Recent advances in epithelium-derived cytokines (IL-33, IL-25 and TSLP) and allergic inflammation. Curr Opin Allergy Clin Immunol *15*, 98–103.

Donskow-Łysoniewska, K., Krawczak, K., and Doligalska, M. (2012). Heligmosomoides polygyrus: EAE remission is correlated with different systemic cytokine profiles provoked by L4 and adult nematodes. Exp. Parasitol. *132*, 243–248.

Druzd, D., Matveeva, O., Ince, L., Harrison, U., He, W., Schmal, C., Herzel, H., Tsang, A.H., Kawakami, N., Leliavski, A., et al. (2017). Lymphocyte Circadian Clocks Control Lymph Node Trafficking and Adaptive Immune Responses. Immunity *46*, 120–132.

Elias, D., Wolday, D., Akuffo, H., Petros, B., Bronner, U., and Britton, S. (2001). Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guerin (BCG) vaccination. Clin. Exp. Immunol. *219*, 219–225.

Elias, D., Akuffo, H., and Britton, S. (2006). Helminthes could influence the outcome of vaccines against TB in the tropics. Parasite Immunol. *28*, 507–513.

Elias, D., Britton, S., Aseffa, A., Engers, H., and Akuffo, H. (2008). Poor immunogenicity of BCG in helminth infected population is associated with increased in vitro TGF-β production. Vaccine *26*, 3897–3902.

Evans, H., and Mitre, E. (2015). Worms as therapeutic agents for allergy and asthma: Understanding why benefits in animal studies have not translated into clinical success. J. Allergy Clin. Immunol. *135*, 343–353.

Faulkner, H., Humphreys, N., Renauld, J.C., Van Snick, J., and Grencis, R. (1997). Interleukin-9 is involved in host protective immunity to intestinal nematode infection. Eur. J. Immunol. *27*, 2536–2540.

Faz Lopez, B., Morales-Montor, J., and Terrazas, L.I. (2016). Role of Macrophages in the Repair Process during the Tissue Migrating and Resident Helminth Infections. Biomed Res. Int. *2016*, 11 pages

Feng, X., Classon, C., Yang, Y., Chan, S., Coquet, J., Rothfuchs, A.G., and Nylén, S. Altered distribution of circulating lymphocyte pool induced by chronic infections cause remodeling of non-draining lymph nodes. PLoS Pathogens. *2018*;14(5):e1007008.

Filbey, K.J., Grainger, J.R., Smith, K.A., Boon, L., Van Rooijen, N., Harcus, Y., Jenkins, S., Hewitson, J.P., and Maizels, R.M. (2014a). Innate and adaptive type 2 immune cell responses in genetically controlled resistance to intestinal helminth infection. Immunol. Cell Biol. *92*, 436–448.

Finney, C.A.M., Taylor, M.D., Wilson, M.S., and Maizels, R.M. (2007). Expansion and activation of CD4+CD25+regulatory T cells in Heligmosomoides polygyrus infection. Eur. J. Immunol. *37*, 1874–1886.

Forbes, E.E., Groschwitz, K., Abonia, J.P., Brandt, E.B., Cohen, E., Blanchard, C., Ahrens, R., Seidu, L., McKenzie, A., Strait, R., et al. (2008). IL-9– and mast cell–mediated intestinal permeability predisposes to oral antigen hypersensitivity. J. Exp. Med. *205*, 897–913.

Ganusov, V. V., and Auerbach, J. (2014). Mathematical Modeling Reveals Kinetics of Lymphocyte Recirculation in the Whole Organism. PLoS Comput. Biol. 2014;10(5):e1003586.

Garrood, T., Lee, L., and Pitzalis, C. (2006). Molecular mechanisms of cell recruitment to inflammatory sites: General and tissue-specific pathways. Rheumatology *45*, 250–260.

Gouÿ De Bellocq, J., Ferté, H., Depaquit, J., Justine, J. Lou, Tillier, A., and Durette-Desset, M.C. (2001). Phylogeny of the Trichostrongylina (Nematoda) inferred from 28S rDNA sequences. Mol. Phylogenet. Evol. *19*, 430–442.

Grainger, J.R., Smith, K.A., Hewitson, J.P., McSorley, H.J., Harcus, Y., Filbey, K.J., Finney, C.A.M., Greenwood, E.J.D., Knox, D.P., Wilson, M.S., et al. (2010). Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-β pathway.

J. Exp. Med. *207*, 2331–2341.

Grencis, R.K., and Worthington, J.J. (2016). Tuft Cells: A New Flavor in Innate Epithelial Immunity. Trends Parasitol. *32*, 583–585.

Gupta, G., Oghumu, S., and Satoskar, A.R. (2014). Mechanisms of Immune Evasion in Leishmaniasis. Adv. Appl. Microbiol. *82*, 1–23.

Hawn, T.R., Day, T.A., Scriba, T.J., Hatherill, M., Hanekom, W.A., Evans, T.G., Churchyard, G.J., Kublin, J.G., Bekker, L.G., and Self, S.G. (2014). Tuberculosis Vaccines and Prevention of Infection. Microbiol. Mol. Biol. Rev. *78*, 650–671.

Hay John B, Hobbs Barry B. (1977). The flow of blood to lymph nodes and its relation to lymphocyte traffic and the immune response. The J Exp Med. 145(1):31-44.

Hotez, P.J., Brindley, P.J., Bethony, J.M., King, C.H., Pearce, E.J., and Jacobson, J. (2008). Helminth infections: the great neglected tropical diseases. J. Clin. Invest. *118*, 1311–1321.

Ikomi, F., Kawai, Y., and Ohhashi, T. (2012). Recent Advance in Lymph Dynamic Analysis in Lymphatics and Lymph Nodes. Ann. Vasc. Dis. *5*, 258–268.

James, C.E., Hudson, A.L., and Davey, M.W. (2009). Drug resistance mechanisms in helminths: is it survival of the fittest? Trends Parasitol. *25*, 328–335.

Janeway, C.A., Travers, P., Walport, M., and Shlomchik, M. (2001). Immunobiology. 884.

Ji, M., Liang, H., Locksley, R.M., Francisco, S., and Francisco, S. (2016). HHS Public Access. *529*, 221–225.

Jia, T.-W., Melville, S., Utzinger, J., King, C.H., and Zhou, X.-N. (2012). Soil-Transmitted Helminth Reinfection after Drug Treatment: A Systematic Review and Meta-Analysis. PLoS Negl. Trop. Dis. *6*, e1621.

Johnston, C.J.C., Smyth, D.J., Kodali, R.B., White, M.P.J., Harcus, Y., Filbey, K.J., Hewitson, J.P., Hinck, C.S., Ivens, A., Kemter, A.M., et al. (2017). A structurally distinct TGF-β mimic from an intestinal helminth parasite potently induces regulatory T cells. Nat. Commun. *8*.

Kane, M.M., and Mosser, D.M. (2001). The Role of IL-10 in Promoting Disease Progression in Leishmaniasis. J. Immunol. *166*, 1141–1147.

Katz, N., Chaves, A., and Pellegrino, J. (1972). A Simple Device for Quantitative Stool Thick-smear Tecnhique in Schistosomiasis Mansoni. Rev. Inst. Med. Trop. Sao Paulo *14*, 397–400.

Kedl, R.M., and Tamburini, B.A. (2015). Antigen archiving by lymph node stroma: A novel function for the lymphatic endothelium. Eur. J. Immunol. *45*, 2721–2729.

Khan, W.I., Richard, M., Akiho, H., Blennerhasset, P.A., Humphreys, N.E., Grencis, R.K., Van Snick, J., and Collins, S.M. (2003). Modulation of intestinal muscle contraction by interleukin-9 (IL-9) or IL-9 neutralization: Correlation with worm expulsion in murine nematode infections. Infect. Immun. *71*, 2430–2438.

King, I.L., and Mohrs, M. (2009). IL-4–producing CD4 $^{+}$ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. J. Exp. Med. *206*, 1001–1007.

King, I.L., Mohrs, K., Meli, A.P., Downey, J., Lanthier, P., Tzelepis, F., Fritz, J.H., Tumanov, A. V, Divangahi, M., Leadbetter, E.A., et al. (2017). Intestinal helminth infection impacts the systemic distribution and function of the naive lymphocyte pool. Mucosal Immunol. *2017* Sep;10(5):1160-1168

Knopp, S., Mohammed, K.A., Speich, B., Hattendorf, J., Khamis, I.S., Khamis, A.N., Stothard, J.R., Rollinson, D., Marti, H., and Utzinger, J. (2010). Albendazole and Mebendazole Administered Alone or in Combination with Ivermectin against Trichuris trichiura : A Randomized Controlled Trial. Clin. Infect. Dis. *51*, 1420–1428.

Lacey, R.W. (1982). Medical Microbiology. Volume 15, No 4.

Lee, H.K., and Iwasaki, A. (2007). Innate control of adaptive immunity: Dendritic cells and beyond. Semin. Immunol. *19*, 48–55.

Liu, Q., Sundar, K., Mishra, P.K., Mousavi, G., Liu, Z., Gaydo, A., Alem, F., Lagunoff, D., Bleich, D., and Gause, W.C. (2009). Helminth infection can reduce insulitis and type 1 diabetes through CD25- and IL-10-independent mechanisms. Infect. Immun. *77*, 5347–5358.

Maizels, R.M., and Smith, K.A. (2011). Regulatory T Cells in Infection. Adv Immunol. *2011*;112:73-136

Maizels, R.M., Hewitson, J.P., Murray, J., Harcus, Y.M., Dayer, B., Filbey, K.J., Grainger, J.R., McSorley, H.J., Reynolds, L.A., and Smith, K.A. (2012a). Immune modulation and modulators in Heligmosomoides polygyrus infection. Exp. Parasitol. *132*, 76–89.

Maizels, R.M., Hewitson, J.P., and Smith, K.A. (2012b). Susceptibility and immunity to helminth parasites. Curr. Opin. Immunol. *24*, 459–466.

Mantovani, A., Biswas, S.K., Galdiero, M.R., Sica, A., and Locati, M. (2013). Macrophage plasticity and polarization in tissue repair and remodelling. J. Pathol. *229*, 176–185.

De Meis, J., Aurélio Farias-De-Oliveira, D., Nunes Panzenhagen, P.H., Maran, N., Villa-Verde, D.M.S., Morrot, A., and Savino, W. (2012). Thymus atrophy and double-positive escape are common features in infectious diseases. J. Parasitol. Res. *2012*.

Meurs, L., Polderman, A.M., Vinkeles Melchers, N.V.S., Brienen, E.A.T., Verweij, J.J., Groosjohan, B., Mendes, F., Mechendura, M., Hepp, D.H., Langenberg, M.C.C., et al. (2017). Diagnosing Polyparasitism in a High-Prevalence Setting in Beira, Mozambique: Detection of Intestinal Parasites in Fecal Samples by Microscopy and Real-Time PCR. PLoS Negl. Trop. Dis. *11*, 1–18.

Min, B. (2018). Spontaneous T cell proliferation: A physiologic process to create and maintain homeostatic balance and diversity of the immune system. Front. Immunol. *Mar 19*;9:547.

Mionnet, C., Sanos, S.L., Mondor, I., Jorquera, A., Laugier, J.P., Germain, R.N., and Bajénoff, M. (2011). High endothelial venules as traffic control points maintaining lymphocyte population homeostasis in lymph nodes. Blood *118*, 6115–6122.

Mishra, P., Palma, M., Bleich, D., Loke, P., and Gause, W. (2014). Systemic impact of intestinal helminth infections. Mucosal Immunol. Jul; 7(4):753-62.

Mohrs, K., Harris, D.P., Lund, F.E., and Mohrs, M. (2005). Systemic Dissemination and Persistence of Th2 and Type 2 Cells in Response to Infection with a Strictly Enteric Nematode Parasite. J. Immunol. *175*, 5306–5313.

Mondor, I., Jorquera, A., Sene, C., Adriouch, S., Adams, R.H., Zhou, B., Wienert, S., Klauschen, F., and Bajénoff, M. (2016). Clonal Proliferation and Stochastic Pruning Orchestrate Lymph Node Vasculature Remodeling. Immunity *45*, 877–888.

Morimoto, M., Morimoto, M., Whitmire, J., Xiao, S., Anthony, R.M., Mirakami, H., Star, R.A., Urban, J.F., and Gause, W.C. (2004). Peripheral CD4 T Cells Rapidly Accumulate at the Host:Parasite Interface during an Inflammatory Th2 Memory Response. J. Immunol. *172*, 2424–2430.

Motran, C.C., Silvane, L., Chiapello, L.S., Theumer, M.G., Ambrosio, L.F., Volpini, X., Celias, D.P., and Cervi, L. (2018). Helminth infections: Recognition and modulation of the immune response by innate immune cells. Front. Immunol. *9*, 1–12.

Mueller, S.N., and Germain, R.N. (2009). the Immune System. *9*, 618–629.

Newlove, T., Guimarães, L.H., Morgan, D.J., Alcântara, L., Glesby, M.J., Carvalho, E.M., and Machado, P.R. (2011). Antihelminthic therapy and antimony in cutaneous leishmaniasis: A randomized, double-blind, placebo-controlled trial in patients co-infected with helminths and leishmania braziliensis. Am. J. Trop. Med. Hyg. *84*, 551–555.

Novianty, S., Dimyati, Y., Pasaribu, S., and Pasaribu, A.P. (2018). Risk Factors for Soil-Transmitted Helminthiasis in Preschool Children Living in Farmland, North Sumatera, Indonesia. J. Trop. Med. *2018*. Article ID 6706413, 6 pages

Nylén, S., Maurya, R., Eidsmo, L., Manandhar, K. Das, Sundar, S., and Sacks, D. (2007). Splenic accumulation of IL-10 mRNA in T cells distinct from CD4 $^{+}$ CD25 $^{+}$ (Foxp3) regulatory T cells in human visceral leishmaniasis. J. Exp. Med. *204*, 805–817.

Obieglo, K., Feng, X., Bollampalli, V.P., Dellacasa-Lindberg, I., Classon, C., Österblad, M., Helmby, H., Hewitson, J.P., Maizels, R.M., Gigliotti Rothfuchs, A., et al. (2016). Chronic Gastrointestinal Nematode Infection Mutes Immune Responses to Mycobacterial Infection Distal to the Gut. J. Immunol. 2016 Mar 1;196(5):2262-71.

Pelly, V.S., Kannan, Y., Coomes, S.M., Entwistle, L.J., Rückerl, D., Seddon, B., MacDonald, A.S., McKenzie, A., and Wilson, M.S. (2016). IL-4-producing ILC2s are required for the differentiation of TH2 cells following Heligmosomoides polygyrus infection. Mucosal Immunol. *9*, 1–11.

Phythian-Adams, A.T., Cook, P.C., Lundie, R.J., Jones, L.H., Smith, K.A., Barr, T.A., Hochweller, K., Anderton, S.M., Hämmerling, G.J., Maizels, R.M., et al. (2010). CD11c depletion severely disrupts Th2 induction and development in vivo. J. Exp. Med. *207*, 2089–2096.

Pullan, R.L., Smith, J.L., Jasrasaria, R., and Brooker, S.J. (2014). Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasites and Vectors *7*, 1–19.

Qi, H., Kastenmüller, W., and Germain, R.N. (2014). Spatiotemporal Basis of Innate and Adaptive Immunity in Secondary Lymphoid Tissue. Annu. Rev. Cell Dev. Biol. *30*, 141–167.

Rausch, S., Huehn, J., Kirchhoff, D., Rzepecka, J., Schnoeller, C., Pillai, S., Loddenkemper, C., Scheffold, A., Hamann, A., Lucius, R., et al. (2008). Functional analysis of effector and regulatory T cells in a parasitic nematode infection. Infect. Immun. *76*, 1908–1919.

Resende Co, T., Hirsch, C.S., Toossi, Z., Dietze, R., and Ribeiro-Rodrigues, R. (2007). Intestinal helminth co-infection has a negative impact on both anti-Mycobacterium tuberculosis immunity and clinical response to tuberculosis therapy. Clin. Exp. Immunol. *147*, 45–52.

Reynolds, L.A., Filbey, K.J., and Maizels, R.M. (2012). Immunity to the model intestinal helminth parasite Heligmosomoides polygyrus. Semin. Immunopathol. *34*, 829–846.

Ridley, D.S., and Hawgood, B.C. (1956). The value of formol-ether concentration of faecal cysts and ova. J. Clin. Pathol. *9*, 74–76.

Rothfuchs, A.G., Egen, J.G., Feng, C.G., Antonelli, L.R. V, Bafica, A., Winter, N., Locksley, R.M., and Sher, A. (2009). In situ IL-12/23p40 production during mycobacterial infection is sustained by CD11bhigh dendritic cells localized in tissue sites distinct from those harboring bacilli. J. Immunol. *182*, 6915–6925.

Ruddle, N.H., and Akirav, E.M. (2010). Secondary lymphoid organs: responding to genetic and environmental cues in ontogeny and the immune response. J. Immunol. *183*, 2205–2212.

Sacks, D.L., and Melby, P.C. (2001). Animal models for the analysis of immune responses to leishmaniasis. Curr. Protoc. Immunol. *Chapter 19*, Unit 19.2.

Sadlova, J., Dvorak, V., Seblova, V., Warburg, A., Votypka, J., and Volf, P. (2013). Sergentomyia schwetzi is not a competent vector for Leishmania donovani and other Leishmania species pathogenic to humans. Parasites and Vectors *6*, 186

Schulz, O., Ugur, M., Friedrichsen, M., Radulovic, K., Niess, J.-H., Jalkanen, S., Krueger, A., and Pabst, O. (2014). Hypertrophy of infected Peyer's patches arises from global, interferon-receptor, and CD69-independent shutdown of lymphocyte egress. Mucosal Immunol. Jul;7(4):892-904.

Setiawan, T., Metwali, A., Blum, A.M., Ince, M.N., Urban, J.F., Elliott, D.E., and Weinstock, J. V. (2007). Heligmosomoides polygyrus promotes regulatory T-cell cytokine production in the murine normal distal intestine. Infect. Immun. *75*, 4655–4663.

Shiow, L.R., Rosen, D.B., Brdičková, N., Xu, Y., An, J., Lanier, L.L., Cyster, J.G., and Matloubian, M. (2006). CD69 acts downstream of interferon-α/β to inhibit S1P1 and lymphocyte egress from lymphoid organs. Nature. Mar 23;440 (7083):540-4.

de Silva, N.R., Brooker, S., Hotez, P.J., Montresso, A., Engeles, D., and Savioli, L. (2003). Soil transmitted helminth infection: updating the global picture. Trends Parasitol. *19*, 547–551.

Silver, Z.A., Kaliappan, S.P., Samuel, P., Venugopal, S., Kang, G., Sarkar, R., and Ajjampur, S.S.R. (2018). Geographical distribution of soil transmitted helminths and the effects of community type in South Asia and South East Asia – A systematic review. PLoS Negl. Trop. Dis. *12*, 7–16.

Smith, K.A., Hochweller, K., Hämmerling, G.J., Boon, L., MacDonald, A.S., and Maizels, R.M. (2011). Chronic helminth infection promotes immune regulation in vivo through

dominance of CD11cloCD103- dendritic cells. J. Immunol. *186*, 7098–7109.

Soderberg, K.A., Payne, G.W., Sato, A., Medzhitov, R., Segal, S.S., and Iwasaki, A. (2005). Innate control of adaptive immunity via remodeling of lymph node feed arteriole. Proc. Natl. Acad. Sci. U. S. A. *102*, 16315–16320.

Sorobetea, D., Svensson-Frej, M., and Grencis, R. (2018). Immunity to gastrointestinal nematode infections. Mucosal Immunol. *11*, 304–315.

Sotgiu, S., Angius, A., Embry, A., Rosati, G., and Musumeci, S. (2008). Hygiene hypothesis: Innate immunity, malaria and multiple sclerosis. Med. Hypotheses *70*, 819–825.

Srivastava, P., Singh, T., and Sundar, S. (2011). Genetic heterogeneity in clinical isolates of Leishmania donovani from India. J. Clin. Microbiol. *49*, 3687–3690.

Stein, J. V., and Nombela-Arrieta, C. (2005). Chemokine control of lymphocyte trafficking: A general overview. Immunology *116*, 1–12.

Stoll, N. R. (1947). This wormy world. J Parasitol. Feb;33(1):1-18.

Strachan, D.P. (1989). Household Size. BMJ Br. Med. J. *299*, 1259–1260.

Tamura, T., Ariga, H., Kinashi, T., Uehara, S., Kikuchi, T., Nakada, M., Tokunaga, T., Xu, W., Kariyone, A., Saito, T., et al. (2004). The role of antigenic peptide in CD4+ T helper phenotype development in a T cell receptor transgenic model. Int. Immunol. *16*, 1691–1699.

Tan, K.W., Yeo, K.P., Wong, F.H.S., Lim, H.Y., Khoo, K.L., Abastado, J.-P., and Angeli, V. (2012). Expansion of Cortical and Medullary Sinuses Restrains Lymph Node Hypertrophy during Prolonged Inflammation. J. Immunol. *188*, 4065–4080.

Tanchot, C., and Rocha, B. (1998). The organization of mature T cell pools. Immunol.Today *19*, 575–579.

Tomura, M., Yoshida, N., Tanaka, J., Karasawa, S., Miwa, Y., Miyawaki, A., and Kanagawa, O. (2008). Monitoring cellular movement in vivo with photoconvertible fluorescence protein "Kaede" transgenic mice. Proc. Natl. Acad. Sci. U. S. A. *105*, 10871–10876.

Turner, J.-E., Morrison, P.J., Wilhelm, C., Wilson, M., Ahlfors, H., Renauld, J.-C., Panzer, U., Helmby, H., and Stockinger, B. (2013). IL-9–mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. J. Exp. Med. *210*, 2951–2965.

Urban, J.F., Katona, I.M., and Finkelman, F.D. (1991a). Heligmosomoides polygyrus: CD4+ but not CD8+ T cells regulate the IgE response and protective immunity in mice. Exp. Parasitol. *73*, 500–511.

Urban, J.F., Katona, I.M., Paul, W.E., and Finkelman, F.D. (1991b). Interleukin 4 is important in protective immunity to a gastrointestinal nematode infection in mice. Proc. Natl. Acad. Sci. U. S. A. *88*, 5513–5517.

Vadlejch, J., Petrtýl, M., Zaichenko, I., Čadková, Z., Jankovská, I., Langrová, I., and Moravec, M. (2011). Which McMaster egg counting technique is the most reliable? Parasitol. Res. *109*, 1387–1394.

Valanparambil, R.M., Segura, M., Tam, M., Jardim, A., Geary, T.G., and Stevenson, M.M. (2014). Production and analysis of immunomodulatory excretory-secretory products from the mouse gastrointestinal nematode Heligmosomoides polygyrus bakeri. Nat. Protoc. *9*, 2740–

2754.

Valitutti, S., Coombs, D., and Dupré, L. (2010). The space and time frames of T cell activation at the immunological synapse. FEBS Lett. *584*, 4851–4857.

Wammes, L.J., Hamid, F., Wiria, A.E., De Gier, B., Sartono, E., Maizels, R.M., Luty, A.J.F., Fillié, Y., Brice, G.T., Supali, T., et al. (2010). Regulatory T cells in human geohelminth infection suppress immune responses to BCG and Plasmodium falciparum. Eur. J. Immunol. *40*, 437–442.

Wammes, L.J., Hamid, F., Wiria, A.E., May, L., Kaisar, M.M.M., Prasetyani-Gieseler, M.A., Djuardi, Y., Wibowo, H., Kruize, Y.C.M., Verweij, J.J., et al. (2016). Community deworming alleviates geohelminth-induced immune hyporesponsiveness. Proc. Natl. Acad. Sci. *113*, 12526–12531.

Whittaker, J.H., Carlson, S.A., Jones, D.E., and Brewer, M.T. (2017). Molecular mechanisms for anthelmintic resistance in strongyle nematode parasites of veterinary importance. J. Vet. Pharmacol. Ther. *40*, 105–115.

WHO, W.H.O. (2013). Sustaining the drive to overcome the global impact of neglected tropical diseases. Second WHO Rep. Neglected Trop. Dis. *3.9*, 67–71.

WHO (2012). World Health Statistics 2012.

WHO (2017). Global Tuberculosis Report 2017.

Williams, A. (2012). Immunology: Mucosal and Body Surface Defences.

Wilson, M.S., Taylor, M.D., O'Gorman, M.T., Balic, A., Barr, T.A., Filbey, K., Anderton, S.M., and Maizels, R.M. (2010). Helminth-induced CD19+CD23hi B cells modulate experimental allergic and autoimmune inflammation. Eur. J. Immunol. *40*, 1682–1696.

Wojciechowski, W., Harris, D.P., Sprague, F., Mousseau, B., Makris, M., Kusser, K., Honjo, T., Mohrs, K., Mohrs, M., Randall, T., et al. (2009). Cytokine-Producing Effector B Cells Regulate Type 2 Immunity to H. polygyrus. Immunity *30*, 421–433.

Wong, T., Hildebrandt, M., Thrasher, S.M., Ph, D., Judith, A., Ph, D., Ahima, R.S., Ph, D., and Wu, G.D. (2007). Divergent metabolic adaptations to intestinal parasitic nematode infection in mice susceptible or resistant to obesity. Gastroenterology *133*, 1979–1988.

World Health, O., and Infections, T.D.R.D.R.G. on H. (2012). Research priorities for helminth infections. World Health Organ. Tech. Rep. Ser. xv–xvii, 1-174, back cover.

Xiwei Zheng, Cong Bi, Marissa Brooks, and D.S.H. (2015). HHS Public Access. Anal Chem. *25*, 368–379.

Yang, C.-Y., Vogt, T.K., Favre, S., Scarpellino, L., Huang, H.-Y., Tacchini-Cottier, F., and Luther, S.A. (2014). Trapping of naive lymphocytes triggers rapid growth and remodeling of the fibroblast network in reactive murine lymph nodes. Proc. Natl. Acad. Sci. *111*, E109–E118.

Yap, P., Du, Z.W., Wu, F.W., Jiang, J.Y., Chen, R., Zhou, X.N., Hattendorf, J., Utzinger, J., and Steinmann, P. (2013). Rapid re-infection with soil-transmitted helminths after triple-dose albendazole treatment of school-aged children in yunnan, people's republic of China. Am. J. Trop. Med. Hyg. *89*, 23–31.

Young, A.J., and Hay, J.B. (1995). Rapid turnover of the recirculating lymphocyte pool in

vivo. Int. Immunol. *7*, 1607–1615.

Zaccone, P., and Cooke, A. (2013). Vaccine against autoimmune disease: Can helminths or their products provide a therapy? Curr. Opin. Immunol. *25*, 418–423.

Zaph Colby, Artis David. (2015). Mucosal Immunology (Fourth Edition) Volume 1, pages 1023-1035.

Zhao, A., McDermott, J., Urban, J.F., Gause, W., Madden, K.B., Yeung, K.A., Morris, S.C., Finkelman, F.D., and Shea-Donohue, T. (2003). Dependence of IL-4, IL-13, and Nematode-Induced Alterations in Murine Small Intestinal Smooth Muscle Contractility on Stat6 and Enteric Nerves. J. Immunol. *171*, 948–954.